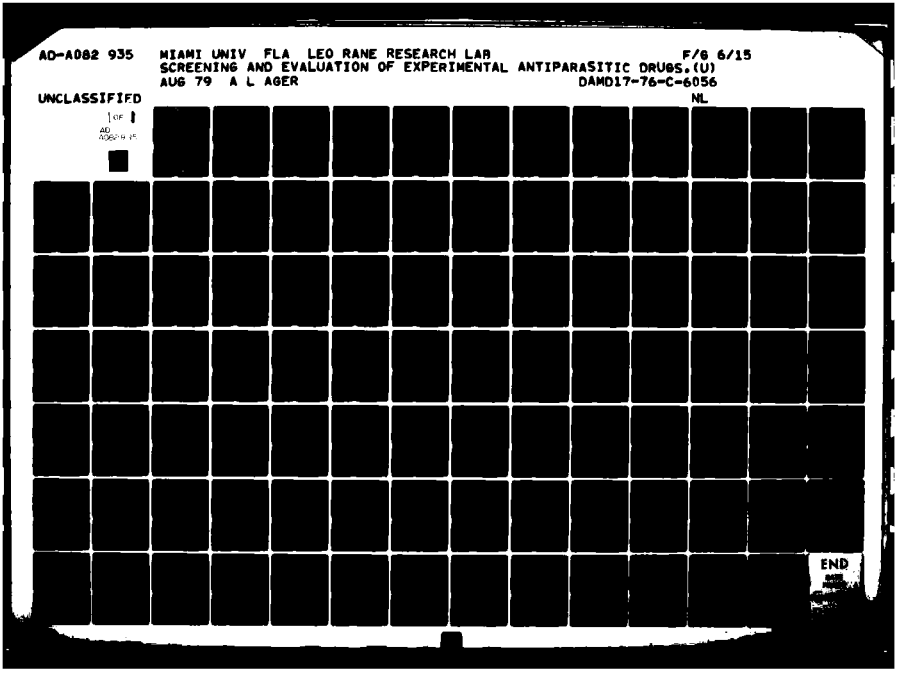
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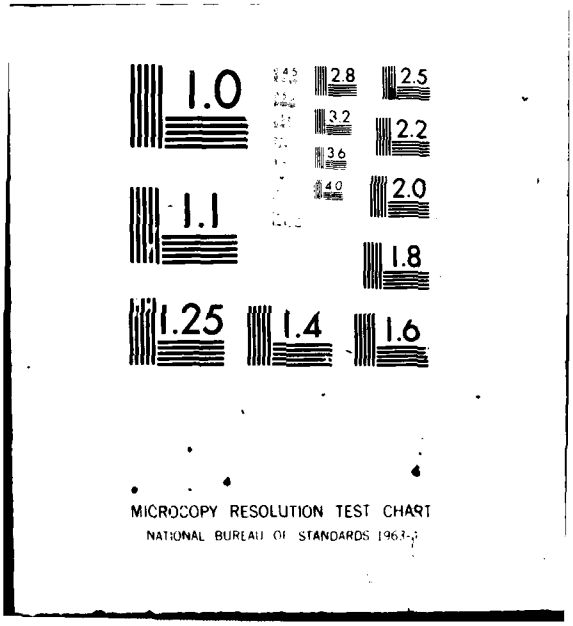
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REPORT NUMBER THIRTEEN

SCREENING AND EVALUATION OF EXPERIMENTAL ANTIPARASITIC DRUGS

ANNUAL REPORT

ARBA L. AGER, JR., Ph.D.

August 1979

(For the Period from 1 October 1977 through 30 September 1978)

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Anopheles stephensi

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The investigations undertaken during this report period included two primary and one secondary drug screening program in malaria, along with a primary and secondary drug screening program in African trypanosomiasis. The malarial system used Plasmodium berghei infected mice and Anopheles stephensi mosquitoes, while the African trypanosomiasis system used Trypanosoma rhodesiense infected mice.

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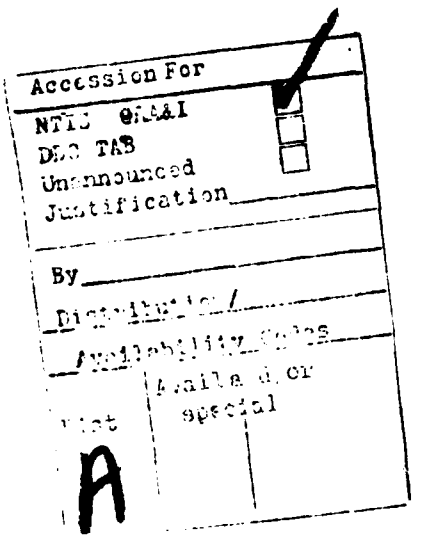
The first primary drug screen in malaria assessed compounds for blood schizonticidal activity. 5,375 three dose level tests were done with 1,261 exhibiting blood schizonticidal activity. The second primary screen in malaria assessed compounds for prophylactic antimalarial activity against sporozoite induced infections. 2,320 three dose level tests were performed to detect activity against sporozoites and/or exoerythrocytic stages. 161 compounds were curative with 116 of these being more active than primaquine. In the secondary drug screening program, 49 compounds were found to be more active than quinine when administered via the oral and subcutaneous routes. The most active compounds were WR 223,146, WR 226,337 and WR 228,710. 86 compounds were tested against one or more of the following lines resistant to chloroquine, cycloguanil, dapsone, mefloquine, pyrimethamine, or quinine, respectively. Two tests were performed to determine if synergistic suppressive activity occured between WR 225,329 plus sulfadiazine and WR 226,337 plus sulfadiazine. Synergistic suppressive activity was found to exist between these compounds and sulfadiazine. 14 compounds were tested for repository blood schizonticidal antimalarial activity for a 17 day period. Four of these compounds retained repository activity for the 17 day period. Five compounds tested for repository activity for a 90 day period were found not to retain activity for this time period. Four compounds tested for a 120 day period were also not repository for this duration of time.

The primary test in African trypanosomiasis evaluated compounds for trypanosomicidal activity against a drug-sensitive line of parasites. 3,032 compounds were tested for trypanosomicidal activity and 91 were found to be active. The secondary test system with African trypanosomiasis involved developing five drug-resistant lines and testing selected compounds against each line. The five lines were resistant to berenil, melarsoprol, pentamidine, stilbamidine and suramin, respectively. In a special study, three new resistant lines were developed: one resistant to stilbamidine, one resistant to WR 163,577, and one resistant to a combination of both compounds. Resistance to stilbamidine developed as fast when given alone as it did when given in combination with WR 163,577. Similarly, resistance to WR 163,577 developed as fast when given alone as it did when given in combination with stilbamidine.



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FOREWORD

In conducting the research described in this Report, the investigator adhered to the principles set forth in the Guide for Care and Use of Laboratory Animals as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute for Laboratory Animal Resources, National Research Council, National Academy of Sciences.

ABSTRACT

The investigations undertaken during this report period included two primary and one secondary drug screening program in malaria, along with a primary and secondary drug screening program in African trypanosomiasis. The malarial system used Plasmodium berghei infected mice and Anopheles stephensi mosquitoes, while the African trypanosomiasis system used Trypanosoma rhodesiense infected mice.

The first primary drug screen in malaria assessed compounds for blood schizonticidal activity. 5,375 three dose level tests were done with 1,261 exhibiting blood schizonticidal activity. The second primary screen in malaria assessed compounds for prophylactic antimalarial activity against sporozoite induced infections. 2,320 three dose level tests were performed to detect activity against sporozoites and/or exoerythrocytic stages. 161 compounds were curative with 116 of these being more active than primaquine. In the secondary drug screening program, 49 compounds were found to be more active than quinine when administered via the oral and subcutaneous routes. The most active compounds were WR 223,146, WR 226,337 and WR 228,710. 86 compounds were tested against one or more of the following lines resistant to chloroquine, cycloguanil, dapsone, mefloquine, pyrimethamine, or quinine, respectively. Two tests were performed to determine if synergistic suppressive activity occured between WR 225,329 plus sulfadiazine and WR 226,337 plus sulfadiazine. Synergistic suppressive activity was found to exist between these compounds and sulfadiazine. 14 compounds were tested for repository blood schizonticidal antimalarial activity for a 17 day period. Four of these compounds retained repository activity for the 17 day period. Five compounds tested for repository activity for a 90 day period were found not to retain activity for this time period. Four compounds tested for a 120 day period were also not repository for this duration of time.

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SUMMARY

Primary and secondary drug screening programs in malaria and African trypanosomiasis for the period of October 1, 1977 to September 30, 1978 are described herein. The malarial system used Plasmodium berghei infected mice and Anopheles stephensi mosquitoes, while the African trypanosomiasis system used Trypanosoma rhodesiense infected mice.

The first primary drug screen in malaria assessed compounds for blood schizonticidal activity. This test consisted of infecting the mice with asexual parasites and administering the drug subcutaneously three days later. The results of drug activity were based upon survival time of treated mice in relation to infected, untreated controls. A drug was considered active if treated mice survived at least twice as long as untreated mice. Mice surviving for 60 days were considered cured. There were 5,375 three dose level tests with 1,261 exhibiting blood schizonticidal activity.

A second primary drug screen in malaria assessed compounds for prophylactic antimalarial activity against sporozoite induced infections. In this test mice were given one subcutaneous injection of drug and four hours later received an intraperitoneal injection of sporozoites isolated from Anopheles stephensi. Prophylactic activity was determined by monitoring mortality daily with drug activity based only upon curative effects. Mice alive for a 30 day period were considered cured. There were 2,320 three dose level tests performed to detect compounds exhibiting curative effects. At least 116 of these compounds were more active than primaquine, and 161 were curative.

In one of the secondary antimalarial test systems compounds were tested against drug-sensitive P-line parasites and one or more drug-resistant lines. Mice were inoculated with asexual parasites on day 0 followed by oral drug administration on days 3, 4 and 5. Blood smears were made on the 6th day and the percentage of cells parasitized and percent suppression of parasitemia were determined. There were 49 compounds tested both orally and subcutaneously against the drug-sensitive line with all 49 exhibiting suppressive activity greater than quinine. The most active compounds were WR 233,146, WR 226,337 and WR 228,710.

There were 86 compounds administered against one or more of the six drug-resistant lines. The number of four level tests against each drug-resistant line was as follows: mefloquine-resistant line (51); chloroquine-resistant line (67); pyrimethamine-resistant line (4); dapsone-resistant line (8); cycloguanil-resistant line (3); quinine-resistant line (16).

Two tests to detect synergistic suppressive activity were done with WR 225,329 plus sulfadiazine and WR 226,337 plus sulfadiazine. Synergistic suppressive activity was observed with 1:1, 2:1, 4:1, 1:4 and 1:2 mixtures of WR 225,329 plus sulfadiazine. Similar synergistic activity was noted with 1: 10.67, 1: 5.33, 1: 2.67 and 1: 1.33 mixtures of WR 226,337 plus sulfadiazine.

The last aspect of the secondary program in malaria involved testing compounds for repository antimalarial activity. Mice were given one subcutaneous injection of drug and challenged either 3, 10 or 17 days later with infected blood. Selected compounds retaining activity for 17 days were further tested for periods of 30, 60, 90 and 120 days. A total of 14 compounds were tested for repository activity of up to 17 days. Four of these compounds retained repository activity for this period. Five compounds were examined for repository activity of 90 days. At the dose levels tested, none of the five exhibited repository activity for the 90 day period. Four compounds tested for 120 day repository activity were found to be negative for this four month period.

A primary screening procedure for the evaluation of trypanosomicidal activity of candidate compounds in Trypanosoma rhodesiense infected mice evaluated a total of 3,032 compounds, 91 of which were recognized as active. In this procedure, groups of mice were infected with trypomastigotes and treated immediately thereafter with one subcutaneous injection of drug. Assessment of activity was made by comparing survival time of treated mice to that of infected, untreated controls. An active compound was one in which treated mice live at least twice as long as untreated animals. Mice surviving for 30 days were considered cured.

The secondary drug screening program in trypanosomiasis included the development and testing of lines of T. rhodesiense resistant to selected trypanosomicidal compounds. Two lines of T. rhodesiense, one with moderate resistance to suramin and one with moderate resistance to melarsoprol developed last year, were placed under increasing drug pressure and made fully resistant to 424 mg/kg of their respective drug. Development of three lines of trypanosomes resistant to berenil, pentamidine and stilbamidine (respectively) has been continued. Several experiments were performed with these five drug-resistant lines to delineate degrees of cross resistance to each of the five compounds as well as to several other active compounds.

A special study designed to develop and test lines resistant to stilbamidine and WR 163,577 (BG 00521) alone and in combination was continued and completed. The WR 163,577 resistant line exhibited greater than a 1000-fold degree of resistance to WR 163,577 while the stilbamidine-resistant line exhibited a 500-fold degree of resistance

to stilbamidine. Parasites of the line resistant to both drugs in combination similarly were greater than a 1000-fold resistant to WR 163,577 and 500-fold resistant to stilbamidine. It appears the development of resistance to either compound is not hindered when the drugs are given in combination as compared to when administered alone.

A SCREENING PROCEDURE FOR ASSESSING THE BLOOD SCHIZONTICIDAL ANTIMALARIAL ACTIVITY OF CANDIDATE COMPOUNDS IN PLASMODIUM BERGHEI INFECTED MICE

The recognition of chloroquine-resistant strains of Plasmodium falciparum in South America and Southeast Asia first posed what is now a critical problem in the chemotherapy of malaria. Parasite resistance to 4-aminoquinolines (e.g., chloroquine and amodiaquine), antifolates (e.g., pyrimethamine) and other standard antimalarial compounds such as quinine, has caused an increased concern for the development of safe alternative therapeutic agents.

The World Health Organization currently estimates that over 100 million cases of malaria worldwide require treatment each year. Recently, chloroquine-resistant parasites have been noted in Africa where over one million children die from malaria yearly. Reports from India, Pakistan, and Sri Lanka indicate a significant resurgence of malaria in that part of the world, with India alone experiencing a rate of approximately 25,000 new cases per day. The current widespread endemicity of malaria and its potential for recurrence in malaria-free zones, the emergence of populations of parasites in Central and South America, Asia and Africa that are resistant to the major available antimalarial agents, and a decrease in vector control programs, emphasize the need for continued mass screening of candidate antimalarial compounds.

A total of 269,146 three level tests were performed from December 1, 1961 through September 30, 1978.

Table I summarizes the compounds tested and the mice used from December 1, 1961 through September 30, 1978.

The test system designed specifically for this operation is based on blood-induced Plasmodium berghei malaria infections in mice. It is a relatively simple and fast procedure. Assessments of antimalarial effect and host toxicity are reproducible and reliable.

All compounds evaluated were obtained from the Department of Medicinal Chemistry at the Walter Reed Institute of Research and included:

(1) Compounds structurally related to chemicals of known value as antimalarial agents.

(2) Compounds structurally unrelated to compounds known to have antimalarial activity.

(3) Structural analogues of compounds found active in our test system and representing several novel chemical groups.

(4) Compounds known to have activity against other infectious disease agents.

Our own breeding colony of ICR/HA Swiss mice has continued to supply the animals used in our tests.

Drug activity was assessed by comparing the maximum survival time of treated malaria-infected animals to the survival time of untreated malaria-infected controls.

Using five and six week old mice and a standard inoculum of P. berghei, it has been possible to produce a consistently uniform disease fatal to 100% of untreated animals within six to seven days.

Since an established disease is less responsive to treatment than a disease in the early stages of development, treatment is withheld deliberately until a fairly high degree of parasitemia is evident. Test compounds are administered subcutaneously in a single dose on the third day post-infection at which time a 10-15% parasitemia has developed. A similar procedure is followed for the oral administration of selected active compounds.

To be classified as active, a compound must suppress the disease and produce an unquestionably significant increase, 100% or more, in the life span of the treated animals over that of the untreated controls. To be considered curative, treated animals must remain alive for 60 days after infection with P. berghei.

The severity of the challenges set up in our test system enhances the reliability of our evaluations and the antimalarial potential of the compounds selected for intensive preclinical studies.

METHOD

ANIMAL HOSTS. The total supply of animals needed to screen candidate compounds has been obtained from our breeding colony of ICR/HA Swiss mice (Mus musculus). Test animals weigh from 18-20 grams; weight variations in any given experimental or control group are carefully limited to within two to three grams. In any given test all animals are approximately the same age.

Animals on test are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib. Once the infected mice are given the drug, they are placed in a room maintained at 84^0 Fahrenheit($\pm 2^0$ F.) and a relative humidity of 66% (±2%).

TEST PROCEDURE. Test animals receive an intraperitoneal injection of approximately 6.8×10^5 parasitized erythrocytes drawn from donor mice infected four days earlier with P. berghei. The donor strain is maintained by passing every four days in separate groups of mice inoculated with 0.5 cc of a 1:50 dilution of heparinized heart blood.

To check factors such as changes in the infectivity of our P. berghei strain or in the susceptibility of the host, one group of mice which serves as the negative control is infected but not treated. In order to determine the effect a drug exerts on a malaria infection two parameters are measured; the first is an increase in survival time, and the second concerns curative action. For comparative purposes, one standard compound, pyrimethamine, is administered at one level (120 mg/kg) to a group of 20 mice. Pyrimethamine serves as a positive control, producing definite increases in survival time and curative effects. Another function of the positive control involves monitoring three procedures: the drug weighing, the preparation of drug solutions and suspensions, and the administration of drugs.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered subcutaneously. Compounds to be administered orally are mixed in an aqueous solution of 0.5% hydroxyethylcellulose - 0.1% tween-80.

Treatment consists of a single dose given subcutaneously or orally three days post-infection. At the time of treatment, a 10-15% parasitemia has developed. Although the disease is well established, it has not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occur before the 6th day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite.

Each compound is initially administered in three graded doses diluted four-fold to groups of five mice per dose level. The top dose is 640, 320 or 160 mg/kg, depending on the amount of compound available for testing. Active compounds are subsequently tested at six or nine dose levels, diluted two-fold from the highest dose. Successive six-level tests are performed at respectively lower doses if necessary until the lower limit of activity is reached.

A drug that is toxic for the host at each of the three levels initially tested is retested at six dose levels diluted two-fold from the lowest toxic dose.

Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice. Treated animals alive at the end of 60 days are considered cured.

DRUG ACTIVITY. Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. An MTD is defined as the highest dose up to 640 mg/kg causing no more than one of five animals to die from drug toxicity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds are rejected after one test, borderline compounds after two tests. Active compounds are characterized by a dose-response curve, which establishes the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose level dilution tests. The total number of active compounds from December 1, 1961 to September 30, 1978 is summarized in Table II.

P. BERGHEI MALARIA IN MICE

TABLE I

SUMMARY OF SCREENING LEVELS

DECEMBER, 1961 - SEPTEMBER, 1978

YEAR	NUMBER OF THREE LEVEL TESTS	NUMBER OF MICE
December, 1961 - May, 1964	6,915	250,000
June, 1964 - May 1965	13,114	215,715
June, 1965 - May, 1966	22,731	350,449
June, 1966 - May, 1967	34,093	531,200
June, 1967 - May, 1968	40,465	636,525
June, 1968 - May, 1969	38,150	603,225
June, 1969 - May, 1970	22,376	411,270
June, 1970 - May, 1971	18,108	322,140
June, 1971 - May, 1972	14,874	262,245
June, 1972 - May, 1973	14,276	231,450
June, 1973 - May, 1974	11,035	168,664
June, 1974 - May, 1975	10,604	168,725
June, 1975 - May, 1976	9,916	155,585
June, 1976 - September, 1977	7,114	123,085
October, 1977 - September, 1978	5,375	82,690
TOTAL	269,146	4,512,968

P. BERGHEI MALARIA IN MICE

TABLE II

SUMMARY OF ACTIVE COMPOUNDS

JUNE 1, 1970 - SEPTEMBER 30, 1978

YEAR	NUMBER OF THREE LEVEL TESTS	NUMBER OF ACTIVE TESTS
June 1, 1970 - May 31, 1971	18,108	805
June 1, 1971 - May 31, 1972	14,874	593
June 1, 1972 - May 31, 1973	14,276	771
June 1, 1973 - May 31, 1974	11,035	394
June 1, 1974 - May 31, 1975	10,604	616
June 1, 1975 - May 31, 1976	9,916	351
June 1, 1976 - Sept. 30, 1977	7,114	1,124
Oct. 1, 1977 - Sept. 30, 1978	5,375	1,261
TOTAL	91,302	5,915

SPOROZOITE INDUCED ANTIMALARIAL TEST IN MICE

Primaquine is the only drug currently used today for causal prophylactic antimalarial activity in humans. This 8-aminoquinoline has two major limitations: First is its poor therapeutic index; and second concerns its involvement in causing hemolytic anemia in persons with a deficiency in glucose 6-phosphate dehydrogenase. New active 8-aminoquinolines as well as other groups of chemicals exhibiting prophylactic activity are needed to combat malaria in the world today.

This test is intended to serve as a primary screening procedure for compounds submitted by the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research.

In this test system, mice receive a subcutaneous injection of drug four hours prior to an intraperitoneal inoculation of sporozoites and survival is monitored for a 30 day period. A similar procedure is followed for the oral administration of selected active compounds. Mice alive after 30 days are considered cured.

METHODS

ANIMALS

Male or female outbred ICR/HA Swiss mice (Mus musculus), six to seven weeks old, weighing 16-17 grams, are used as test animals. They are maintained in groups of 5 and fed water and feed ad lib.

Mice used as a source of gametocytes (donor mice) are eight weeks old and weigh 25-30 grams.

MOSQUITO COLONY

Anopheles stephensi are reared in an insectary maintained at 80^0F and 70% relative humidity with 14 hours of light and 10 hours of darkness. Larvae are fed a solution of 2.5% liver powder once a day. Emerged adults are fed a 10% glucose solution ad lib.

INFECTED MICE AS A SOURCE OF GAMETOCYTES

Donor mice to be used as a source of gametocytes are injected intraperitonially with a dilution of infected heart blood from mice previously infected with sporozoites of Plasmodium berghei.

INFECTION OF MOSQUITOES

Mosquitoes are placed in a room maintained at 70^0F and 70% relative humidity prior to the infected blood meal. Donor mice harboring a 5-20% parasitemia are anesthetized with Nembutal and placed on top of the mosquito cages for one hour to allow the mosquitoes to feed on infected blood. A second infected blood meal is given the following day and thereafter the mosquitoes are maintained on a 10% glucose solution with normal blood meals given at seven day intervals.

ISOLATION OF SPOROZOITES

On the 17th day after the infected blood meal, the mosquitoes are anesthetized with ether, collected in a plastic bag, and weighed. Two and one-half ml. of 0.9% saline plus two and one-half ml. of inactivated mouse plasma are injected into the bag containing the mosquitoes. The contents of this bag are then macerated on a cold table with a teflon plunger. Saline and mouse plasma (1:1) are added to the homogeneous mass on the basis of the weight of the mosquitoes and the dilution desired. The uniform suspension is then filtered to remove legs, wings, tissue and exoskeleton fragments of the mosquitoes. The filtered sporozoite suspension is further diluted until there are approximately 250,000 sporozoites per 0.2 ml. of inoculum.

ADMINISTRATION OF TEST COMPOUNDS

Each compound is ground with a mortar and pestle and then suspended in 0.5% hydroxyethylcellulose - 0.1% tween-80 to make the desired drug doses. The percent free base of each compound is not determined. Four hours prior to the inoculation of sporozoites, compounds are administered either subcutaneously or orally at three graded doses diluted four-fold (160, 40, and 10 mg/kg). Groups of five mice per dose level are used. Subsequent tests employing successive lower four-fold dilutions are made if mice are cured at 10 mg/kg, until the lower limit of a compound's activity is reached.

Deaths that occur before the seventh day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite. A drug that is toxic for the host at each of the three initial dose levels is retested at doses diluted four-fold from 10 mg/kg.

INOCULATION OF MICE WITH SPOROZOITES

Mice are injected intraperitoneally with approximately 250,000 sporozoites. Twenty of these mice are divided into two groups of ten each. One group receives no drug and serves as a negative control. The other group is treated with WR 181,023 (125 mg/kg) and acts as a positive control. One additional group of five infected mice, serving as a treated control, is treated with chloroquine (100 mg/kg).

DETERMINATION OF ANTIMALARIAL ACTIVITY

After the mice have been inoculated with sporozoites, they are placed in a room maintained at 84°F and 66% relative humidity. Antimalarial activity is determined by monitoring mortality daily. Mice alive after 30 days are considered cured.

RESULTS

CONTROLS

Mice inoculated with sporozoites but receiving no drug (negative control group) all routinely die within 7 to 12 days, as do mice receiving chloroquine. Mice serving as positive controls survive for the duration of the experiment (30 days).

COMPOUNDS TESTED AND DRUG ACTIVITY

In the first 178 experiments, 1,684 three-level tests were performed using over 29,810 mice. A total of 831 different compounds were tested at least once. Many were tested several times either orally, subcutaneously, or via both routes.

2,320 three-level tests were performed using over 43,725 mice from October 1, 1977 to September 30, 1978. A total of 1,300 different compounds were tested at least once and many were tested several times either subcutaneously, orally, or via both routes.

For a compound to be considered active, cures must be produced (i.e., survivors for 30 days) in at least two out of five mice at the highest tolerated drug level tested.

In the first 178 experiments there were 99 compounds which were active both subcutaneously and orally. 162 compounds were active only when administered subcutaneously and 144 compounds were active only via the oral route of administration. At least 149 compounds were more active than primaquine.

In the period from October 1, 1977 to September 30, 1978 there were 117 compounds which were active both subcutaneously and orally. 23 compounds were active only when administered subcutaneously and 5 compounds were active only via the oral route of administration. 15 compounds tested only subcutaneously were active while 1 tested only orally was active. At least 116 compounds were more active than primaquine.

SECONDARY ANTIMALARIAL SCREENING SYSTEM

Current prospects for the control of human malaria have been complicated by the occurrence of drug-resistant parasites. Such resistance falls into three categories, namely:

1. Resistance to antifolic drugs (pyrimethamine, chloroguanide, etc.)
2. Resistance to 4-aminoquinolines and acridines (chloroquine, atebrine, quinine, etc.)
3. A combination of 1 and 2 which is referred to as multiple resistance.

Collectively, the several types of resistance impair the effectiveness of all major suppressive drugs. Hence, a great need exists for alternative drugs as well as new combinations of drugs.

New candidate compounds are emerging from a primary blood schizonticidal screening program, and it is particularly important to determine quite early which of the new candidates are likely to be useful against the various types of drug-resistant malaria. Experience has indicated that plasmodia of animals can be used for this purpose.

The specific aims of this test system were to conduct a sequential battery of chemotherapeutic studies in Plasmodium berghei infected mice on active compounds (discreet or open) emerging from the Department of Defense - sponsored screening programs in order to determine which substances were worthy of further consideration as potential agents for dealing with drug-resistant malaria.

METHODS

The techniques used in this secondary drug testing program fell into two categories, namely:

1. Studies designed to determine if a new agent was likely to be useful against the various types of drug-resistant malaria, and
2. General chemotherapeutic characterization of selected new agents to suggest optimal methods of use and specific purposes they may serve.

The testing was done with Plasmodium berghei in outbred ICR/HA female Swiss mice (Mus musculus) weighing 20-25 grams. Briefly, this testing entailed procedures for the direct assessment of the effects of drugs on the parasitemia. Various gross tolerance observations were also recorded which served as guides indicating the usefulness of the new test agents as drugs for treatment of malaria.

More specifically, activities included elucidation of the apparent mode of action of agents by testing them in parallel against drug-sensitive P. berghei (KBG-173) and various drug-resistant derivatives

of this malaria strain. The 6 drug-resistant derivatives included a chloroquine-resistant, a cycloguanil-resistant, a dapsone-resistant, a mefloquine-resistant, a pyrimethamine-resistant and a quinine-resistant line.

TEST DESIGN

When a new compound is obtained it is subjected to a battery of testing procedures, the extent of which depends on its degree of activity in suppressing murine malaria infections. The first test procedure is a 6-day suppressive test against the drug-sensitive P-line.

If the compound is active against the P-line, then a 6-day test against one or more drug-resistant lines follows. In this basic 6-day test, mice are divided into groups of 7 and inoculated with parasites intraperitoneally. Drugs are administered twice a day, usually orally, in a volume of 10 ml/kg on the third, fourth and fifth days after inoculation of parasites. All drugs are mixed in aqueous 0.5% hydroxyethylcellulose-0.1% tween-80 and ultrasonicated when necessary. Drug doses are prepared using 100% of the free base of each drug. One group of ten infected mice receives the vehicle alone and serves as a negative control. Thin blood films and final group weights are taken on the sixth day after inoculation of parasites. Microscope examination of Giemsa-stained blood smears is made to determine the percentage of cells parasitized, percent suppression of parasitemias, and significance values for the suppression of parasitemias. Significance values are based on a calculation of the percent suppression of parasitemia which is determined by comparing the parasitemia of each treated mouse with the mean parasitemia of the negative controls. Drug tolerance is reflected by the percent weight change and the proportion of mice that survive treatment. Toxicity is attributed to drug action when a -14% or greater weight change occurs or when one or more mice die before the blood smears are taken.

REGULAR P-LINE TESTING

Each new drug is tested first against the drug-sensitive P-line usually via both the oral and subcutaneous routes of administration. The drug dosages for the first test are normally 64, 16, 4 and 1 mg/kg/day for three days. If less than a 90% suppression of the parasitemia (SD_{90}) is obtained with the lower dose of 1 mg/kg/day, then testing at lower doses is performed. Chloroquine is tested as a reference against the P-line at levels of 2, 3 and 4 mg/kg/day. A quinine index (Q) is calculated by comparing the SD_{90} value obtained from the chloroquine dose response curve and the SD_{90} value of the new compound:

$$Q = \frac{SD_{90} \text{ of chloroquine}}{SD_{90} \text{ of new compound}} \times 30$$

DRUG-RESISTANT LINES

Compounds that suppress the P-line parasitemia by at least 90% with 64 mg/kg or less are subjected to testing against one or more of the six drug-resistant lines. These lines include a chloroquine-resistant, a cycloguanil-resistant, a dapsone-resistant, a mefloquine-resistant, a pyrimethamine-resistant and a quinine-resistant line. The amount of testing against the resistant lines depends upon the structure of each new compound as it relates to the structure of known antimalarials. A maximum dose of 256 mg/kg/day is administered orally along with doses of 64, 16 and 4 mg/kg/day.

ESTIMATES OF POTENCY AND CROSS RESISTANCE

Doses required for a given degree of effect, such as 90% suppression or SD_{90}, are estimated graphically from plots made on log-probit paper. The ratios of the SD_{90} (or whatever other level of effect, e.g., SD_{70} or SD_{50}) are used to delineate the degree of cross resistance (Tables I and II).

SYNERGISTIC AND/OR ANTAGONISTIC SUPPRESSIVE TEST WITH DRUG COMBINATIONS

When two drugs are administered at the same time to an established infection of malaria, one of three things can result with regard to the ensuing parasitemia: an additive suppressive effect; a greater than additive suppressive effect (potentiation or synergism); or a less than additive suppressive effect (antagonism). A synergistic suppressive effect appears to be most pronounced when the compounds involved have related but different modes of action. For example, sulfonamides and pyrimethamine inhibit the metabolism of the parasites at different sequential steps along the same biochemical pathway of folic acid. Sulfonamides block para-aminobenzoic acid from being incorporated into folic acid while pyrimethamine inhibits dihydrofolic acid reductase which is responsible for the conversion of dihydrofolic to tetrahydrofolic acid.

In order to test for synergistic or antagonistic suppressive activity, the two drugs are administered either alone or as a mixture by gavage twice daily on days three, four and five after the mice are infected via the intraperitoneal route. The effects are determined from parasitemia counts of blood smears made one day after completion of treatment.

RESULTS

A total of fourty-nine different compounds were tested against the P-line. Fourty-two of these were also tested against one or more drug-resistant lines. Fourty-four other compounds were tested against only one or more drug-resistant lines.

REGULAR DRUG-SENSITIVE P-LINE

Each of the fourty-nine different compounds were tested via the oral and subcutaneous routes of administration. They were all found to be active via both routes and each was more active than quinine. The most active compounds tested with quinine equivalent values exceeding 1000 were WR 223,146, WR 226,337 and WR 228,710.

DRUG-RESISTANT LINES

The number of 4 level tests used with each drug-resistant line are as indicated below:

Line	Type of Resistance	No. of 4 Level Tests
A	Mefloquine-Resistance	51
C	Chloroquine-Resistance	67
M	Pyrimethamine Resistance	4
S	Dapsone-Resistance	8
T	Cycloguanil-Resistance	3
U	Quinine-Resistance	16

TESTS TO DETERMINE SYNERGISTIC SUPPRESSIVE ACTIVITY

Two experiments were performed to detect whether synergistic activity occured between sulfadiazine and WR 225,329 and sulfadiazine and WR 226,337. Synergism was observed in all of the following drug combinations:

MIXTURES	RATIO OF MIXTURES
WR 225,329 + Sulfadiazine	1:1
"	2:1
"	4:1
"	1:4
"	1:2
WR 226,337 + Sulfadiazine	1:10.67
"	1:5.33
"	1:2.67
"	1:1.33

TABLE I

SUMMARY OF DATA ON COMPOUNDS TESTED
OCTOBER 1, 1977 - SEPTEMBER 30, 1978

WR COMPOUND NO./ BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
448 AX 09212	190						200			P.O.	-
1544	191								>256[a]	P.O.	-
AR 20613	192			140[b]						P.O.	-
2976 AW 23860	191								>256[a]		-
25979	173				180					P.O.	>3
AH 78744	192				200					P.O.	-
99210	195	3	23							P.O.	>2
AU 20967			<1							S.C.	-
102,796	178				<4					P.O.	-
BG 78778	181				0.45					P.O.	-
	189								0.35	P.O.	>731
	191								0.72	P.O.	-
	192			0.66						P.O.	-
150,012	179	>78	<1							P.O.	>64
BE 17348			<1							S.C.	-
150,015	155	>93	<1							P.O.	>16, <64
BH 13149			<1							S.C.	-
	163	276	0.38							P.O.	42
			0.3							S.C.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q^1	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
150,017	156	>90	<1							P.O.	>16, <64
BH 30097			<1							S.C.	-
	163	262	0.4							P.O.	40
			0.5							S.C.	-
	175				1					P.O.	-
	189								<0.25	P.O	-
	190						0.24			P.O.	-
154,923	173				1.8					P.O.	>33
BH 14020											
155,004	156	47	1.9							P.O.	8
BH 13158			1.5							S.C.	
BH 30104	189								1.3	P.O.	>12
	190						5.8			P.O.	-
169,626	195	4	21							P.O.	>3
AX 97007			<1							S.C.	-
169,627	195	9	8.6							P.O.	>7
AX 97016			<1							S.C.	-
181.023	192			2.2						P.O.	>2
BE 50003											
194,965	191								>256[a]	P.O.	>73
BG 33940	192			>256[a]						P.O.	-
219,774	191								>256[a]	P.O.	>182
BH 35903	194	57	1.4							P.O.	-
			2.4							S.C.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q^1	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
221,036	166	344	0.27							P.O.	>59
BG 00478			<0.25							S.C.	-
	177				2					P.O.	-
	193			1.9						P.O.	-
223,146	167	>102	<1							P.O.	>16, <64
BH 30999			<1							S.C.	-
	168	>1151	<0.06							P.O.	>258
			<0.06							S.C.	-
	192			0.12						P.O.	
224,097	166	12	7.6							P.O.	>8
BH 35154			2.2							S.C.	-
	177				32					P.O.	-
225,448	154	>288	<0.25							P.O.	>256
BH 35761			<0.25							S.C.	-
	188			3.3						P.O.	-
	189								2.8	P.O.	-
	190						2.9			P.O.	-
226,337	167	>1645	<0.06							P.O.	>258
BH 30980			<0.06							S.C.	-
	175				2.1					P.O	-
	189								<0.25	P.O.	-
	190						2.4			P.O.	-
	191								0.2[b]	P.O.	-
	192			1						P.O.	-
226,899	162	>81	<1							P.O.	26
BG 52623			<1							S.C.	-
	168	600	0.15							P.O.	-
			0.16							S.C.	-
	188			0.37						P.O.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
226,970	173				<4					P.O.	1
BH 13532	186				1.8					P.O.	-
	190						0.7			P.O.	-
	193			<4						P.O.	-
228,258	151					0.78		0.96		P.O.	>533
BH 38968	167	212	0.48							P.O.	-
			0.6							S.C.	-
	177				50[b]					P.O.	-
	193			90[b]						P.O.	-
228,710	156	>90	<1							P.O.	50
BG 66412			<1							S.C	-
	163	1312	0.08							P.O.	-
			0.13							S.C.	-
	173				0.15					P.O.	-
	193			0.2						P.O.	-
229,049	151					12.5		11		P.O.	>5
BG 85319	191								>64[a]	P.O.	-
229,090	151					30[b]				P.O.	>1
BG 85686											
229,403	193			120						P.O.	>4
BG 71002											
229,404	193			200						P.O.	>4
BG 71011											
229,561	191								>32[a]	P.O.	3
BG 72901											

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
230,083 BG 78985	161			27[a]						P.O.	>10
230,084 BG 78976	161			76[a]						P.O.	>11
230,190 BG 85373	151					11				P.O.	-
230,386	151							>16[a]		P.O.	ND
BG 81624	161			35						S.C.	18*Done S.C.
230,687	180	6	13							P.O.	>4
BH 50400			<1							S.C.	-
	181				8					P.O.	-
231,030 BG 89077	170			2.3						P.O.	>16
231,133	153			>256[a]						P.O.	>94
BG 89139	161			>256[a]						P.O.	-
231,134	153			230[b]						P.O.	>91
BG 89157	161			>256[a]						P.O.	-
231,135 BG 89200	170			<4						P.O.	>77
231,158	169	67	1.15							P.O.	>64
BG 89148			3.9							S.C.	-
231,159 BG 89273	161			>256[a]						P.O.	>106

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q^1	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
231,160 BG 89120	161			160[a]						P.O.	>365
231,350 BG 94630	153			40						P.O.	>25
	165				14					P.O.	-
	170				>16[b]					P.O.	-
231,530 BG 94916	165				2.9					P.O.	-
	170			6.2						P.O.	>64
231,623 BG 94836	172				7					P.O.	>88
231,624 BG 94827	172				<4					P.O.	>88
	186				2.7					P.O.	-
231,627 BG 94845	172				9.8					P.O.	N.D.
231,628 BG 94818	172				<4					P.O.	>102
	186				2.6					P.O.	-
232,584 BH 05361	162	>81	<1							P.O.	>82
			<1							S.C.	-
	168	136	0.66							P.O.	-
			0.78							S.C.	-
	173				2.7					P.O.	-
	174			0.45						P.O.	-
232,708 BH 07776	153			>256[a]						P.O.	>88
	172				<4					P.O.	-
	186				2.6					P.O.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
232,716	194	70	1.15							P.O.	>13
BH 78711			0.62							S.C.	-
232,745	153			250						P.O.	>5
BH 07801											
232,956	153			<4						P.O.	>256
BH 08773	172				2					P.O.	-
233,078	170			0.54						P.O.	>50
BH 08764	172				1.2					P.O.	-
233,124	153			35						P.O.	>21
BH 09118	172				43					P.O.	-
233,152	152	2	33							P.O.	>3
BH 09396			12.5[b]							S.C.	-
	173				40.7					P.O.	-
	188			62						P.O.	-
233,153	152	7	10							P.O.	>12
BH 09403			10							S.C.	-
	173				30					P.O.	-
	188			27						P.O.	-
233,154	152	3	28							P.O.	>9
BH 09387			13							S.C.	-
	178				3					P.O.	-
	188			23.5[b]						P.O.	-
233,316	155	22	4.2							P.O.	>60
BH 10728			11							S.C.	-
	175				39					P.O.	-
	184				<4					P.O.	-
	186				9					P.O.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
233,325 BH 10657	174			15						P.O.	20
233,335 BH 10648	174			<4						P.O.	>91
233,339 BH 10586	155	54	1.7 3.7							P.O. S.C.	>37 -
	175				>64[a]					P.O.	-
233,340 BH 10611	162	7	12 30							P.O. S.C	>21 -
	174			80[a]						P.O.	-
233,342 BH 10700	170 178			170 12.5						P.O. P.O.	>28 -
233,343 BH 10719	170 178			130	6.6					P.O. P.O.	>25 -
233,344 BH 10693	174 178			10[a]	7.2					P.O. P.O.	>23 -
233,346 BH 10602	174			46						P.O.	>182
233,348 BH 10595	174			60.6[b]						P.O.	20
233,537 BH 12964	173 174			1.4	2.6					P.O. P.O.	>33 -
233,545 BH 13345	158	34	2.7 2.8							P.O. S.C.	1 -
	177				3					P.O.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
233,600	194	32	2.5							P.O.	>1
BH 50624			2.6							S.C.	-
233,602	158	>93	<1							P.O.	>102
BH 13434			1.2							S.C.	-
	175				<4					P.O.	-
BH 50615	178				<4					P.O.	-
	181				1.6					P.O.	-
BH 13434	184				0.45					P.O.	-
	188			1.2						P.O.	-
BH 50615	194	32	2.5							P.O.	-
			2.7							S.C.	-
233,630	179	>78	<1							P.O.	>256
BH 50277			<1							S.C.	-
	181				<4					P.O.	-
233,637	169	43	1.8							P.O.	>142
BH 49596			2.9							S.C.	-
	178				120[b]					P.O.	-
	189								>256[a]	P.O.	-
	193			5.6[b]						P.O.	-
234,059	154	>72	<1							P.O.	>256
BH 17736			<1							S.C.	-
	175				42					P.O.	-
	184				0.66					P.O.	-
	188			19						P.O.	-
234,095	159	6	12.5							P.O.	>20
BH 23869			12.5							S.C.	-
	175				>256[a]					P.O.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
234,578	159	39	2.1							P.O.	>121
BH 27590			3.4							S.C.	-
	175				9.4					P.O.	-
	177				5					P.O.	-
	189								11.25	P.O.	-
	190						6.2			P.O.	-
234,579	158	42	2.2							P.O.	1
BH 27072			2.8							S.C.	-
	177				1.7					P.O.	-
234,732	159	25	3.3							P.O.	>77
BH 27385			11							S.C.	-
	177				35[b]					P.O.	-
235,325	177				26					P.O.	N.D.
BH 35056											
235,476	179	>78	<1							P.O.	>256
BH 35216			<1							S.C.	-
	181				115					P.O.	-
	193			>256						P.O.	-
235,485	154	>288	<0.25							P.O.	>256
BH 35770			<0.25							S.C.	-
	165				3.3					P.O.	-
	188			2						P.O.	-
	189								2.6	P.O.	-
	190						2.8			P.O.	-
235,662	180	>102	<1							P.O.	>256
BH 35949			<1							S.C.	-
	181				150[b]					P.O.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
235,721	169	17	4.5							P.O.	>14
BH 36375			11							S.C.	-
	178				7.2[a]					P.O.	-
	192			>64[a]						P.O.	-
235,728	185	20	5.2							P.O.	>51
BH 38182			13.5							S.C.	-
	186				6.8					P.O.	-
235,732	185	>108	<1							P.O.	>256
BH 38226			<1							S.C.	-
	186				>256[a]					P.O.	-
235,768	185	>108	<1							P.O.	>16, <64
BH 38333			2.4							S.C.	-
	186				<1					P.O.	-
235,960	180	>78	<1							P.O.	>256
BH 39027			1.6							S.C.	-
	181				<16[b]					P.O.	-
	183	>81	<1							P.O.	-
			<1							S.C.	-
	184				<4					P.O.	-
235,961	183	81	1.25							P.O.	>51
BH 39045			<1							S.C.	-
235,962	183	65	1.6							P.O.	>64
BH 38995			1							S.C.	-
	184				2					P.O.	-
236,332	182	>102	<1							P.O.	>256
BH 48026			3.5							S.C.	-
	184				6[b]					P.O.	-

TABLE I
(continued)

WR COMPOUND NO. / BOTTLE NO.	Exp. No.	Q[1]	SD_{90} (mg/kg/day)[2] P	A	C	M	S	T	U	R_x[3]	T.I.[4]
236,336 BH 48044	182	>102	<1							P.O.	>256
			2.8							S.C.	-
	184				175					P.O.	-
236,337 BH 48035	182	>102	<1							P.O.	>256
			<1							S.C.	-
	184				6.8[a]					P.O.	-

[1] Quinine Index = potency relative to quinine against sensitive parasites (P-line) via the oral route of administration.

[2] Amount of drug to suppress 90% of the parasites for the following lines: P = drug sensitive; A = Mefloquine-resistant; C = Chloroquine-resistant; M = Pyrimethamine-resistant; S = Dapsone-resistant; T = Cycloguanil-resistant; U = Quinine-resistant.

[3] P.O. = Oral. S.C. = Subcutaneous.

[4] Therapeutic Index.

[5] ND = Not Determined due to lack of activity of compound.

*a = Comparison point at SD_{50}.

@b = Comparison point at SD_{70}.

TABLE II

Degrees of Cross Resistance with the Six Drug-Resistant Lines of *Plasmodium berghei*

COMPOUND NO./ BOTTLE NO.	Exp. No.	CROSS RESISTANCE[1] A	C	M	S	T	U
1544	191						>121[a]
AR 20613	192	58[b]					
25979	173		2				
AH 78744	192		2				
102,796	178		N.D.				
BH 78778	181		N.D.				
	189						N.D.
	191						N.D.
	192	N.D.					
150,017	175		2				
BH 30097	189						0
	190				0		
154,923	173		0				
BH 14020							
155,004	189						0
BH 30104	190				3		
181,023	192	2					
BE 50003							
194,965	191						>106[a]
BG 33940	192	>106[a]					
219,774	191						>256[a]
BH 35903							
221,036	177		7				
BG 00478	193	7					
223,146	192	0					
BH 30999							
224,097			4				
BH 35154							
225,448	188	>13					
BH 35761	189						>11
	190				>11		
226,337	175		>33				
BH 30980	189						0
	190				>38		
	191						3[b]
	192	>16					

TABLE II
(continued)

COMPOUND NO./ BOTTLE NO.	Exp. No.	CROSS RESISTANCE[1] A	C	M	S	T	U
226,899 BG 52623	188	2					
226,970	173		0				
BH 13532	186		0				
	190				0		
	193	0					
228,258	151			0		0	
BH 38968	177		217[b]				
	193	188[b]					
228,710	173		0				
BG 66412	193	2					
229,049	151			0		0	
BG 85319	191						>8[a]
229,090	151			0			
BG 85686							
229,403	193	4					
BG 71002							
229,404	193	3					
BG 71011							
229,561	191						>5[a]
BG 72901							
230,083	161	4[a]					
BG 78985							
230,084	161	11[a]					
BG 78976							
230,190	151			0			
BG 85373							
230,386	151					>4[a]	
BG 81624	161	10					
230,687	181		0				
BH 50400							
231,030	170	2					
BG 89077							
231,133	153	>121[a]					
BH 89139	161	>196[a]					
231,134	153	96[b]					
BG 89157	161	>170[a]					

TABLE II
(continued)

COMPOUND NO./ BOTTLE NO.	Exp. No.	CROSS RESISTANCE[1] A	C	M	S	T	U
231,135 BG 89200	170	0					
231,159 BG 89273	161	>1024[a]					
231,160 BG 89120	161	432[a]					
231,350	153	4					
BG 94630	165		0				
	170		>1[b]				
231,530	165		2.9				
BG 94916	170	6					
231,623 BG 94836	172		2				
231,624	172		0				
BG 94827	186		0				
231,627 BG 94845	172		N.D.				
231,628	172		0				
BG 94818	186		0				
232,584	173		4				
BH 05361	174	0					
232,708	153	>124[a]					
BH 07776	172		0				
	186		0				
232,745 BH 07801	153	5.7					
232,956	153	N.D.					
BH 08773	172		>2				
233,078	170	0					
BH 08764	172		0				
233,124	153	2					
BH 09118	172		3				
233,152	173		0				
BH 09396	188	2					

TABLE II
(continued)

COMPOUND NO. / BOTTLE NO.	Exp. No.	CROSS RESISTANCE[1] A	C	M	S	T	U
233,153	173		3				
BH 09403	188	2					
233,154	178		0				
BH 09387	188	>9					
233,316	175		9				
BH 10728	184		0				
	186		2				
233,325	174	4					
BH 10657							
233,335	174	0					
BH 10648							
233,339	175		>37[a]				
BH 10586							
233,340	174	38[a]					
BH 10611							
233,342	170	18					
BH 10700	178	0					
233,343	170	13					
BH 10719	178		0				
233,344	174	4[a]					
BH 10693	178		0				
233,346	174	32					
BH 10602							
233,348	174	25[b]					
BH 10595							
233,537	173		0				
BH 12964	174	0					
233,545	177		0				
BH 13345							
233,602 BH 13434	175		0				
BH 50615	178		0				
	181		0				
BH 13434	184		0				
	188	0					
233,630	181		N.D.				
BH 50277							

TABLE II
(continued)

COMPOUND NO. / BOTTLE NO.	Exp. No.	CROSS RESISTANCE[1] A	C	M	S	T	U
233,637	178		>120[b]				
BH 49596	189						>256[a]
	193	>5[b]					
234,059	175		>42				
BH 17736	184		0				
	188	>19					
234,095	175		>36[a]				
BH 23869							
234,578	175		4				
BH 27590	177		2				
	189						5
	190				3		
234,579	177		0				
BH 27072							
234,732	177		14[b]				
BH 27385							
235,325	177		N.D.				
BH 35056							
235,476	181		>115				
BH 35216	193	>256					
235,485	165		>13.2				
BH 35770	188	>8					
	189						>10
	190				>11		
235,662	181		>150[b]				
BH 35949							
235,721	178		4.5[a]				
BH 36375	192	>40[a]					
235,728	186		0				
BH 38182							
235,732	186		>256[a]				
BH 38226							
235,768	186		N.D.				
BH 38333							
235,960	181		>16[b]				
BH 39027	184		N.D.				

TABLE II
(continued)

COMPOUND NO. / BOTTLE NO.	Exp. No.	CROSS RESISTANCE[1] A	C	M	S	T	U
235,962 BH 38995	184		0				
236,332 BH 48026	184		>6[b]				
236,336 BH 48044	184		>175				
236,337 BH 48035	184		>6.8[a]				

[1] Cross resistance value obtained by comparison at SD_{90} with the following drug-resistant lines:

A = Mefloquine-resistant
C = Chloroquine-resistant
M = Pyrimethamine-resistant
S = Dapsone-resistant
T = Cycloguanil-resistant
U = Quinine-resistant

[a] Cross resistance value obtained by comparisons at SD_{50}.

[b] Cross resistance value obtained by comparisons at SD_{70}.

A SCREENING PROCEDURE FOR ASSESSING THE REPOSITORY ANTIMALARIAL ACTIVITY OF CANDIDATE COMPOUNDS IN PLASMODIUM BERGHEI INFECTED MICE

An effective, reliable screening program is essential for the development of single dose antimalarial drugs that are protective for prolonged periods of time. In over ten years of research and development, only a few drugs have shown promise as repository antimalarials (acedapsone and cycloguanil in particular). These have found limited use in the field due to such factors as the ease with which resistance to some of the compounds is induced, the variable drug sensitivity of Plasmodium species, and the local discomfort that may be produced upon administration of the drug.

The screening program described herein permits a determination of the repository activity of large numbers of compounds in different vehicles. The experimental design for such a program is based on tests of several standard antimalarials and new drugs found active in this laboratory's primary antimalarial screening tests. These drugs are mixed in an aqueous or oil-based vehicle and administered to mice. Mice are subsequently challenged at various time intervals with Plasmodium berghei infected erythrocytes to test for repository activity.

All compounds evaluated are obtained from the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research.

Animals used in the tests are supplied by our breeding colony of ICR/HA Swiss mice.

Compounds displaying curative activity in preliminary repository tests are further evaluated for long term repository activity by extending the length of time between drug administration and challenge with P. berghei to periods of up to four months.

METHODS

ANIMALS

Male or female ICR/HA Swiss mice (Mus musculus) of uniform age and weight are used. They are placed in a room maintained at 75°F (±2°F) and a relative humidity of 66% (±2%). Mice are housed in groups of five and fed water and feed ad lib.

VEHICLES

Compounds tested for repository activity are mixed in either of two vehicles:

1. Aqueous 0.5% hydroxyethylcellulose - 0.1% tween-80 (HEC).
2. Refined peanut oil (oil).

TEST PROCEDURE

On day zero, mice are given a single subcutaneous or oral dose of the compound. Negative controls receive injections of the vehicle alone and routinely die six to eight days after challenge with parasites. On days +3, +10, and +17, treated and control subgroups are challenged in parallel with approximately 5.0×10^5 parasitized erythrocytes obtained from P. berghei infected donor mice. Mice are challenged only once.

Selected compounds that have displayed activity when administered 17 days prior to challenge are further tested using a similar procedure with challenges on days +30, +60, and +90 or on day +120.

Mortality over a four week period is used as an index of compound repository activity. Negative controls all die six to nine days after the challenge. Deaths occuring before the 6th day post-infection are attributed to compound toxicity. Treated animals alive four weeks after challenge with P. berghei are considered cured.

COMPOUNDS

The compounds tested, dose levels and route of administration are summarized below. The percent-free base of each compound is considered in calculating doses.

Compounds Administered Subcutaneously
3, 10, and 17 days Prior to Challenge*

Compound	Dose (mg/kg)
Acridine	
WR 234,059 (BH 17736)	32
4-Aminoquinoline	
WR 219,774 (BE 79759)	40
WR 228,258 (BG 85640)	40
WR 231,133 (BG 89139)	48
WR 231,134 (BG 89157)	64
WR 233,124 (BH 09118)	40

Compound	Dose (mg/kg)
8-Aminoquinoline	
WR 225,448 (BH 35761)	40
WR 231,350 (BG 94630)	48
WR 232,956 (BH 08773)	40
WR 235,485 (BH 35770)	40
Quinazoline	
WR 232,745 (BH 07801)	48
Sulfone	
DADDS (acedapsone)	400
Triazine	
WR 5,473 (AU 76138 Cycloguanil pamoate)	400
Other	
WR 232,708 (BH 07776)	32

* Reported in Antimalarial Repository Drug Screening Test #7.

Compounds Administered Subcutaneously
30, 60 and 90 days Prior to Challenge†

Compound	Dose (mg/kg)
Phenol	
WR 194,965 (BG 56327)	320
4-Aminoquinoline	
WR 219,774 (BH 35903)	40
WR 225,449 (BG 94925)	40
WR 228,258 (BH 38968)	40
WR 228,979 (BH 08326)	40

† Reported in Antimalarial Repository Drug Screening Test #8.

Compounds Administered Subcutaneously
120 days Prior to Challenge∞

Compound	Dose (mg/kg)
4-Aminoquinoline	
WR 219,774 (BE 79759)	40
WR 228,258 (BG 85640)	40

Compound	Dose (mg/kg)
Sulfone	
DADDS	400
Triazine	
WR 5,473 (AU 76138 Cycloguanil pamoate)	400

∞ Reported in Antimalarial Repository Drug Screening Test #7.

RESULTS

Results are summarized in Tables I through III. As a guide to interpreting the tables, the following scale is used to assess the relative activity of each compound:

No. Mice Cured/Total	Activity
0/5	None
1/5	Slight
2/5 or 3/5	Moderate
4/5	High
5/5	Complete

CONCLUSION

Compounds Administered 3, 10 and 17 Days Prior to Challenge (Tables I and II)

Compounds demonstrating completely curative repository activity at the specified doses through the 17-day challenge include two 4-aminoquinolines (WR 219,774 and WR 228,258) and the triazine, cycloguanil pamoate (WR 5,473). The sulfone, DADDS, was highly active. The remaining compounds, listed below, did not retain blood schizonticidal activity for 17 days when administered subcutaneously at the specified doses:

WR 225,448	WR 232,745
WR 231,133	WR 232,956
WR 231,134	WR 233,124
WR 231,350	WR 234,059
WR 232,708	WR 235,485

Compounds Administered 30, 60 and 90 Days Prior to Challenge (Table III)

The 4-aminoquinoline WR 219,774 (40 mg/kg) exhibited slight repository activity through the 60-day challenge when administered subcutaneously in oil and complete activity through the 30-day challenge in HEC. WR 194,965, WR 225,449, WR 228,258 and WR 228,979 displayed no greater than slight repository activity through the 30-day challenge in either vehicle.

Compounds Administered 120 Days Prior to Challenge

None of the compounds tested for repository activity of 120 days retained blood schizonticidal activity for that period when administered subcutaneously at the specified doses. These compounds are listed below:

WR 5,473	WR 228,258
WR 219,774	DADDS

TABLE I

The Curative Effects of Antimalarial Compounds Administered Subcutaneously to Mice 3, 10 and 17 Days Prior to Challenge* With Plasmodium Berghei

Compound**	Dose (mg/kg)	No. Mice Cured*** 3 Day Challenge	10 Day Challenge	17 Day Challenge
(-) Control	0	0	0	0
Acridine				
WR 234,059	32	0	0	0
4-Aminoquinoline				
WR 219,774	40	5	5	5
WR 228,258	40	5	5	5
WR 231,133	48	2	0	0
WR 231,134	64	0	0	0
WR 233,124	40	0	0	0
8-Aminoquinoline				
WR 225,448	40	5	1	0
WR 231,350	48	0	0	0
WR 232,956	40	0	0	0
WR 235,485	40	2	0	0
Quinazoline				
WR 232,745	48	0	0	0
Sulfone				
DADDS	400	4	4	4
Triazine				
WR 5,473 (Cycloguanil pamoate)	400	5	5	5
Other				
WR 232,708	32	2	0	0

* Each mouse challenged only once with 5.0×10^5 parasitized erythrocytes. Five mice per challenge group.

** Compounds mixed in aqueous 0.5% hydroxyethylcellulose - 0.1% tween-80.

*** Alive four weeks after infection with P. berghei.

TABLE II

Daily Mortality and Cures of Mice Challenged* with Plasmodium berghei 3, 10 and 17 Days After Subcutaneous Administration of Antimalarial Compounds

Compound**	Dose (mg/kg)	Challenge (Days after Rx)	Challenge Results (No. Deaths/Day Post-Infection)	No. Cures***
(-) Control	0	3	1/6, 4/7	0
		10	1/6, 3/7, 1/8	0
		17	4/7, 1/8	0
WR 5,473	400	3	-	5
		10	-	5
		17	-	5
WR 219,774	40	3	-	5
		10	-	5
		17	-	5
WR 225,448	40	3	-	5
		10	1/10, 2/11, 1/16	1
		17	4/7, 1/8	0
WR 228,258	40	3	-	5
		10	-	5
		17	-	5
WR 231,133	48	3	1/13, 1/16, 1/18	2
		10	3/7, 1/9, 1/10	0
		17	4/7, 1/8	0
WR 231,134	64	3	1/6, 2/8, 1/13, 1/14	0
		10	4/7, 1/8	0
		17	2/7, 2/8, 1/9	0
WR 231,350	48	3	2/6, 2/8, 1/11	0
		10	4/7, 1/8	0
		17	4/7, 1/8	0
WR 232,708	32	3	1/7, 1/8, 1/9	2
		10	5/7	0
		17	4/7, 1/8	0
WR 232,745	48	3	5/7	0
		10	3/7, 2/8	0
		17	4/7, 1/8	0
WR 232,956	40	3	1/6, 1/13, 2/18, 1/22	0
		10	3/7, 2/8	0
		17	2/7, 3/8	0

TABLE II
(continued)

Compound**	Doses (mg/kg)	Challenge (Days after Rx)	Challenge Results (No. Deaths/Day Post-Infection)	No. Cures***
WR 233,124	40	3	3/7, 2/8	0
		10	2/6, 2/7, 1/8	0
		17	4/7, 1/8	0
WR 234,059	32	3	4/7, 1/8	0
		10	4/7, 1/8	0
		17	3/7, 2/8	0
WR 235,485	40	3	1/10, 1/11, 1/16	2
		10	1/6, 3/7, 1/8	0
		17	3/7, 2/8	0
DADDS	400	3	1/16	4
		10	1/24	4
		17	1/6	4

* Each mouse challenged only once with 5.0×10^5 parasitized erythrocytes. Five mice per challenge group.

** Compounds mixed in aqueous 0.5% hydroxyethylcellulose - 0.1% tween-80.

*** Alive four weeks after infection with P. berghei.

TABLE III

The Curative Effects of Selected Compounds Administered Subcutaneously 30, 60 and 90 Days Prior to Challenge* with Plasmodium Berghei

Compound	Dose** (mg/kg)	Vehicle***	No. Mice Cured† 30-Day Challenge	60-Day Challenge	90-Day Challenge
Phenol					
WR 194,965	320	HEC	0	0	0
		Oil	1	0	0
4-Aminoquinolines					
WR 219,774	40	HEC	5	0	0
		Oil	1	1	0
WR 225,449	40	HEC	1	0	0
		Oil	0	0	0
WR 228,258	40	HEC	1	0	0
		Oil	1	0	0
WR 228,979	40	HEC	0	0	0
		Oil	1	0	0

* Each mouse challenged once with 5.0×10^5 parasitized erythrocytes. Five mice per challenge group.

** Percent-free base considered in calculating doses.

*** HEC - aqueous 0.5% hydroxyethylcellulose - 0.1% tween-80.
Oil - Refined peanut oil.

† Alive four weeks after challenge with P. berghei.

A SCREENING PROCEDURE FOR THE EVALUATION OF TRYPANOSOMICIDAL ACTIVITY OF CANDIDATE COMPOUNDS IN TRYPANOSOMA RHODESIENSE INFECTED MICE

The test system described herein was developed specifically to evaluate the trypanosomicidal activity of large numbers of candidate compounds. Based on blood-induced Trypanosoma rhodesiense infections in mice, it acts as a primary screen or as a secondary screen and confirmatory test and gives precise quantitative evaluations of chemical compounds that demonstrate potentially useful therapeutic and/or prophylactic activity in T. rhodesiense infections. Consequently, it is also a helpful guideline in the synthesis of new active agents.

All candidate compounds were obtained from the Department of Medicinal Chemistry at the Walter Reed Institute of Research and included:

1. Chemicals structurally related to compounds of known value in the treatment or prevention of T. rhodesiense infections;

2. Chemicals structurally unrelated to compounds of known value in the treatment or prevention of T. rhodesiense infections;

3. Structural analogues of compounds that have demonstrated activity in our screening procedure and represent novel chemical groups;

4. Compounds known to have activity against other infectious agents.

Table I summarizes the number of compounds tested and the number of mice used from August 1, 1972 through September 30, 1978.

Our own colony of ICR/HA Swiss mice provided all the test animals needed in this operation. Using mice of a given age, sex and weight and a standard inoculum of the Wellcome CT-strain of T. rhodesiense, it has been possible to produce a consistently uniform disease fatal to 100% of untreated animals within 4 to 6 days.

Test compounds were administered subcutaneously in a single dose on the day of infection. Selected active compounds were administered orally.

Drug activity was assessed by comparing the maximum survival time of the treated trypanosome-infected animals to the survival time of the untreated trypanosome-infected control animals. To be classified as active, a compound must suppress the disease and produce an increase of at least 100% in the life span of the treated animals over that of the untreated controls. Treated animals must remain alive for 30 days for a compound to be considered curative.

METHODS

ANIMAL HOSTS

ICR/HA Swiss mice (Mus musculus) used in this screening procedure weigh 30 to 32 grams with weight variations in any given experimental or control group carefully limited to three grams. In all tests the animals are of the male sex and approximately the same age.

Animals are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib. Once the mice are drugged, they are kept in a room maintained at a temperature of 84^0F ($\pm 2^0$F) with a relative humidity of 66% (±2%).

TEST PROCEDURE

Test animals receive an intraperitoneal injection of 1.5 cc of a 1:50,000 dilution of heparinized heart blood drawn from a donor mouse infected three days earlier.

The donor line is maintained by three-day blood passes: Each animal receives 0.1 cc of a 1:500 dilution of heparinized heart blood drawn from a three-day donor. Donors, like test animals, weigh 30 to 32 grams; weight variations for each pass are limited to three grams.

One group of infected, untreated mice are included as a negative control to check factors such as changes in the infectivity of our T. rhodesiense strain or in the susceptibility of the host. In order to determine the effect a drug exerts on a trypanosome infection, two parameters are measured: the increase in mouse survival time and drug curative action. For comparative purposes, two standard compounds, stilbamidine isethionate and 2-hydroxystilbamidine isethionate, are administered at one level each (26.5 mgs/kg) to separate groups of ten mice. These diamidines serve as positive controls, producing definite increases in survival time and curative effects. Another function of the two positive controls involves a check on whether three procedures are performed correctly: The drug weighing; the preparation of drug solutions and suspensions; and the administration of drugs.

DRUG ADMINISTRATION

Test compounds are dissolved or suspended in peanut oil before they are administered subcutaneously. Compounds to be administered orally are mixed in an aqueous solution of 0.5% hydroxyethylcellulose - 0.1% tween-80.

Treatment consists of a single dose given subcutaneously or orally two to three hours after the injection of parasites. Deaths that occur before the fourth day, when untreated controls begin to die, are regarded as a result of action by the drug, not parasites.

Each compound is initially administered in three graded doses diluted four-fold to groups of five mice per dose level. The top dose is 424, 212, or 106 mg/kg, depending on the amount of compound available for testing. Active compounds are subsequently tested at six or nine dose levels, diluted two-fold from the highest dose. Successive six-level tests are performed at respectively lower doses if necessary until the lower limit of activity is reached.

A drug that is toxic for the host at each of the three levels initially tested is retested at six dose levels diluted two-fold from the lowest toxic dose.

DRUG ACTIVITY

Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. An MTD is defined as the highest dose up to 424 mg/kg causing no more than one of five animals to die from drug toxicity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds are rejected after one test; borderline compounds after two tests. Active compounds are characterized by a dose-response curve, which establishes the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose level dilution tests. Treated animals alive at the end of 30 days are considered cured.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST TRYPANOSOMA RHODESIENSE INFECTIONS IN MICE

The screening procedure was developed and its reliability established during the initial stages of this project which was from June 1, 1972 through May 1, 1973. 3,030 selected compounds were screened; including those agents known to be effective against T. rhodesiense infections and drugs drawn from our antimalarial program. 68 of these demonstrated a degree of activity sufficient to produce at least 100% increases in the survival time of treated T. rhodesiense infected mice.

1,581 compounds were tested in the period during June 1, 1973 through May 31, 1974. 185 of these demonstrated a degree of activity sufficient to produce at least 100% increases in the survival time of treated T. rhodesiense infected mice: 92 were active subcutaneously and 93 were active orally.

1,826 compounds were tested in the period of June 1, 1974 through May 31, 1975. 298 compounds were recognized as active: 225 of these were active subcutaneously and 73 were active orally.

1,653 compounds were tested during the period of June 1, 1975 through May 31, 1976. 257 compounds were recognized as active: 198 were active subcutaneously and 59 were active orally.

4,235 compounds were tested from June 1, 1976 to September 30, 1977. 396 compounds were recognized active: 109 were active both orally and subcutaneously; 17 were active only orally; 270 were active only subcutaneously.

Compounds tested during the period from October 1, 1977 through September 30, 1978 numbered 3,032. 91 were recognized as active compounds: 14 of these were active both orally and subcutaneously; 9 were active only orally; 54 were active only subcutaneously (refer to Table I).

The activity evaluations provided by our screening procedure are precise and quantitative, therefore the above breakdowns are significant when considering the following:

a) The dose-response curves of active compounds administered subcutaneously reveal a wider spread between the MTD and the MED than those of active compounds administered orally;

b) These dose-responses also display a wider spread of toxic effects when active compounds toxic for the host are administered subcutaneously rather than orally.

TABLE I

COMPOUNDS TESTED AND MICE UTILIZED
August 1, 1972 - September 30, 1978

DATE	Number of 3 Level Tests	Number of Mice
August 1, 1972 - May 31, 1973	3,030	51,405
June 1, 1973 - May 31, 1974	1,581	25,360
June 1, 1974 - May 31, 1975	1,826	33,850
June 1, 1975 - May 31, 1976	1,653	30,290
June 1, 1976 - Sept. 30, 1977	4,235	73,280
Oct. 1, 1977 - Sept. 30, 1978	4,025	64,600
TOTAL	16,350	278,785

DRUG-RESISTANT TRYPANOSOME LINES

The resistance of Trypanosoma rhodesiense to selected antitrypanosomal compounds can be induced by repeated drug pressure in an in vivo test system. This is achieved by infecting mice with a standard inoculum of parasites, administering the test compound in a dose just below the curative level, and passing parasites from these animals to a new set of mice when the parasitemia rises to a desirable level. Passes are made every three to four days with drug doses being increased as resistance develops at each dose level.

This type of study can establish the rate at which T. rhodesiense acquires resistance in mice to selected compounds. Cross resistance to trypanosomicidal compounds found to be active against the drug-sensitive line in primary screening tests may also be determined.

Lines of trypanosomes have been developed which are completely or partially resistant to the following compounds:

Completely Resistant	Partially Resistant
Melarsoprol	Berenil
Suramin	Pentamidine
	Stilbamidine

METHODS

ANIMALS

Male or female ICR/HA Swiss mice (Mus musculus) of approximately the same age and weight are used in all procedures. Animals are housed in groups of five, fed a standard laboratory diet and given water ad lib. Mice are kept in a room maintained at 84° F(±2°F) and a relative humidity of 66% (±2%).

DEVELOPMENT AND MAINTENANCE OF DRUG-RESISTANT LINES

On day 0, fifteen male or female mice are divided into three groups of five animals. All animals are initially inoculated intraperitoneally with drug-sensitive T. rhodesiense (Wellcome CT-strain) trypomastigotes in saline-diluted blood (1:500) drawn from a previously infected donor mouse. Group I serves as a negative control, receiving no drug. Group II receives drug either orally or subcutaneously on day 0 and day 1. Group III is given the same dose of drug by the same route on day 0 only.

On day 3 or 4, fifteen new mice are infected with saline-diluted blood (1:500) from Group II. The pass is made from Group III if Group II animals demonstrate no parasites upon blood examination. These newly infected mice are similarly divided into three groups and given the same drug regimen as that just described. Passes are thus made every three or four days from the most recently infected and treated groups of animals. Drug doses are increased as resistance develops.

Once complete resistance to the highest tolerated dose of the compound is reached, the line is passed two times each week using two groups of five mice. Group I mice receive no drug and serve as a negative control. Group II mice receive a low dose of drug to maintain drug pressure and serve as donor mice for the next pass.

TEST PROCEDURE - INOCULATION OF PARASITES

Giemsa-stained blood smears from donor mice infected three days earlier with T. rhodesiense trypomastigotes are microscopically examined to determine parasitemias (i.e., number of trypomastigotes in a field of 100 erythrocytes). One set of test animals is infected with the drug-sensitive line of parasites and receives an intraperitoneal injection of 0.5cc of a 1:50,000 dilution of heparinized heart blood drawn from a donor mouse harboring a parasitemia of 30-35%. Other sets of mice are similarly infected with each drug-resistant line to be tested. Blood dilutions are made such that all mice infected with the resistant lines receive approximately the same number of trypomastigotes as mice infected with the drug-sensitive line.

One group of ten infected mice from the sensitive line and from each resistant line serve as negative controls, receiving no drug.

TEST PROCEDURE - COMPOUND ADMINISTRATION

Test compounds are mixed in either peanut oil for subcutaneous administration or 0.5% hydroxyethylcellulose - 0.1% tween-80 for oral administration. Compounds are given immediately following challenge with trypomastigotes.

Compound doses are diluted two or four-fold from a level that has been projected to be fully curative (should sufficient quantities be available). Five mice are used for each dose level.

COMPOUND ACTIVITY

Mortality is used as an index of drug activity. Untreated negative control mice routinely die on days 4 or 5 after inoculation of parasites. Increases in life span relative to that of negative controls at each dose level are recorded. Curative activity is used in assessing the level of resistance of selected compounds. Mice surviving for 30 days are considered cured.

The CD_{50} (minimal dose curing at least three of five mice) is used as a basis for establishing levels of resistance and determining compound cross resistance. A comparison of CD_{50} values for each compound when tested against the drug-sensitive and drug-resistant lines determines the degree of resistance or cross resistance (degree of resistance = CD_{50} of resistant line ÷ CD_{50} of sensitive line). A cross resistance of four-fold or less will not be considered significant in this report as compounds were often administered at four-fold dilutions. The spread produced by such dilutions is too great using the CD_{50} as an index of activity to attach significance to an apparent cross resistance of four-fold. A cross resistance greater than four-fold is considered significant.

RESULTS

DEVELOPMENT OF RESISTANCE TO MELARSOPROL

Resistance to this arsenical has been progressively induced and maintained over a period of 11 months of drug pressure and 85 line passages as illustrated in Table I.

The melarsoprol-resistant line demonstrated a 64-fold degree of resistance to melarsoprol (CD_{50} = 26.5 mg/kg) in Experiment #13 after a period of one and a half months of drug pressure and 16 line passages (Tables II-IV).

This line demonstrated greated than a 256-fold degree of resistance to melarsoprol in Experiment #15 (Tables V-VIII). Following a period of seven months of drug pressure and 59 line passages, the melarsoprol-resistant line displayed complete resistance to melarsoprol when this drug was administered at a dose of 424 mg/kg.

CROSS RESISTANCE WITH MELARSOPROL

The melarsoprol-resistant line demonstrated a 4-fold degree of cross resistance to suramin in Experiment #15 (Tables V-VIII).

DEVELOPMENT OF RESISTANCE TO SURAMIN

Resistance to this naphthylamine sulfonate has been progressively induced and maintained over a period of nearly 22 months and 206 line passages, as illustrated in Table IX.

The suramin-resistant line demonstrated greater than a 256-fold degree of resistance to suramin (CD_{50} > 424 mg) in Experiment #15 after a period of 18 months of drug pressure and 180 line passages. This line was thus shown to be completely resistant to suramin when the drug was administered at a dose of 424 mg/kg (Tables V-VIII).

CROSS RESISTANCE WITH SURAMIN

The suramin-resistant line exhibited a 16-fold degree of cross resistance to melarsoprol in Experiment #15 (Tables V-VIII).

DEVELOPMENT OF RESISTANCE TO BERENIL

Resistance to this diamidine has been progressively induced over a period of nearly 14 months of drug pressure and 132 line passages, as illustrated in Table X.

The berenil-resistant line demonstrated a 256-fold degree of resistance to berenil (CD_{50} = 26.5 mg/kg) in Experiment #9 after a period of one and a half months and 15 line passages (Tables XI-XIII).

A 512-fold degree of resistance to berenil (CD_{50} = 53 mg/kg) was demonstrated in Experiment #11 after a period of two months and 22 line passages (Tables XIV-XVI).

A 256-fold degree of resistance to berenil (CD_{50} = 106 mg/kg) was demonstrated in Experiment #14 after a period of six months and 61 line passages (Tables XVII-XX).

CROSS RESISTANCE WITH BERENIL

The cross resistance levels of several trypanosomicidal compounds with berenil as demonstrated by the berenil-resistant line are shown below. All compounds were administered subcutaneously.

Compound	Experiment No.*	Cross Resistance with Berenil
Stilbamidine	9	16-fold
	11	128-fold
	14	32-fold
Suramin	9	1-fold
Melarsoprol	9	64-fold
	11	16-fold
WR 163,577 (BG 00521)	9	>128-fold
	11	>128-fold
Pentamidine	11	16-fold

* Experiment #9 = Tables XI-XIII
Experiment #11 = Tables XIV-XVI
Experiment #14 = Tables XVII-XX.

DEVELOPMENT OF RESISTANCE TO PENTAMIDINE

Resistance to this diamidine has been progressively induced over a period of two and a half months of drug pressure and 25 line passages, as illustrated in Table XXI.

DEVELOPMENT OF RESISTANCE TO STILBAMIDINE

Resistance to this diamidine was progressively induced during a period of over 14 months of drug pressure and 145 line passages, as indicated in Table XXII. The stilbamidine-resistant line was discontinued at line passage number 145 and development of a new line resistant to stilbamidine was initiated shortly thereafter. The development of this new line's resistance to stilbamidine over a period of five months of drug pressure and 48 line passages is illustrated in Table XXIII.

The first stilbamidine-resistant line demonstrated a 32-fold degree of resistance to subcutaneously administered stilbamidine (CD_{50} = 212 mg/kg) in Experiment #14 after a period of nearly 14 months of drug pressure and 138 line passages (Tables XVII-XX). The CD_{50} for stilbamidine administered orally against this line was greater than 212 mg/kg.

CROSS RESISTANCE WITH STILBAMIDINE

The first stilbamidine-resistant line demonstrated a 260-fold degree of cross resistance to berenil in Experiment #14 (Tables XVII - XX).

DEVELOPMENT OF RESISTANCE TO STILBAMIDINE AND WR 163,577 ALONE AND IN COMBINATION

It is a well established fact in malarial infections that the development of resistance to compounds can be drastically reduced or completely blocked if they are administered in combination rather than alone. Based upon this rationale a similar type of experiment was designed for T. rhodesiense parasites. Three new lines of T. rhodesiense were developed: one resistant to stilbamidine, one resistant to WR 163,577, and one resistant to a combination of stilbamidine and WR 163,577. The objectives for this work were two-fold: first to compare the rates of acquisition of resistance to WR 163,577 vs. stilbamidine, and second to determine if the development of resistance would occur at a different rate when both compounds were administered in combination.

The experimental design was similar to that described above for the development of other trypanosome-resistant lines. Drug levels used in the continuation of line passages to induce resistance to each drug alone and in combination are tabulated in Table XXIV. The WR 163,577-resistant line demonstrated greater than a 1,000-fold degree of resistance to WR 163,577 while the stilbamidine-resistant line exhibited a 500-fold degree of resistance to stilbamidine (Tables XXV-XXVIII). Parasites of the line resistant to both drugs in combination similarly displayed greater than a 1,000-fold degree of resistance to WR 163,577 and a 500-fold degree of resistance to stilbamidine.

Resistance to WR 163,577 developed to a greater degree than resistance to stilbamidine by the 16th passage of each line.

It appears the development of resistance to either compound is not hindered when the drugs are given in combination.

TABLE I

DEVELOPMENT OF A MELARSOPROL-RESISTANT LINE OF TRYPANOSOMA RHODESIENSE

Passage Number	Dose (mg/kg)*
1-7	0.14
8	0.28
9-10	0.41
11-12	0.55
13-14	1.1
15-22	5.5
23-26	8.2
27-28	10.9
29-40	32.8
41-58	100
59-85**	20

* Drug administered subcutaneously.

** Beginning with passage number 59, line passed twice weekly and given the indicated drug dosage to maintain drug resistance.

TABLE II

Experiment #13

COMPOUND CD_{50}* VALUES AND DEGREE OF RESISTANCE**
OF THE MELARSOPROL-RESISTANT LINE OF
TRYPANOSOMA RHODESIENSE TO MELARSOPROL

Drug	CD 50 (mg/kg) Sensitive Line	Melarsoprol-Resistant Line	Degree of Resistance of Melarsoprol-Resistant Line
Melarsoprol BE 80510	0.414	26.5	64-fold

* CD 50 is the minimal dose curing at least 3 of 5 mice.

** Degree of resistance = CD 50 of resistant line ÷ CD 50 of sensitive line.

NOTE: Drug administered subcutaneously.

TABLE III

Experiment #13

CURATIVE ACTIVITY OF MELARSOPROL AT VARIOUS DOSES WHEN TESTED AGAINST THE DRUG-SENSITIVE AND MELARSOPROL-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Drug	Dose (mg/kg)	GROUP NO. & (NO. MICE CURED*) Sensitive Line	Melarsoprol-Resistant Line
(-) Control	0	1 (0)	6 (0)
Melarsoprol BG 80510	106	-	7 (5)
	26.5	-	8 (4)
	6.63	2 (5)	9 (0)
	1.66	3 (5)	10 (0)
	0.41	4 (3)	11 (0)
	0.1025	5 (1)	12 (0)

* No. mice alive 30 days after infection with T. rhodesiense.

TABLE IV

Experiment #13

DAILY MORTALITY OF MICE TREATED WITH MELARSOPROL FOLLOWING INFECTION WITH THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Group No.	No. mice dead/day died	No. mice cured
1	5/4	0
2	-	5
3	-	5
4	2/11	3
5	1/6, 2/7, 1/8	1
6	5/4	0
7	-	5
8	1/18	4
9	3/6, 2/7	0
10	2/4, 3/5	0
11	4/4, 1/6	0
12	4/4, 1/5	0

TABLE V

Experiment #15

THE CD_{50}* VALUES OF MELARSOPROL AND SURAMIN ADMINISTERED SUBCUTANEOUSLY AND TESTED AGAINST THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

	CD_{50} (mg/kg)		
Drug	Sensitive Line	Melarsoprol - Resistant Line	Suramin - Resistant Line
Melarsoprol	1.66	>424	26.5
Suramin	1.66	6.63	>424

* CD_{50} is the minimal dose curing at least 3 of 5 mice.

TABLE VI

Experiment #15

DEGREES OF RESISTANCE* OF DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE TO MELARSOPROL AND SURAMIN

Drug	Degree of Resistance	
	Melarsoprol - Resistant Line	Suramin - Resistant Line
Melarsoprol	>256	16
Suramin	4	>256

* Degree of Resistance = CD_{50} of resistant line ÷ CD_{50} of sensitive line.

Note: Drugs administered subcutaneously.

TABLE VII

Experiment #15

CURATIVE ACTIVITY OF MELARSOPROL AND SURAMIN AT VARIOUS DOSES WHEN TESTED AGAINST THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Drug	Dose (mg/kg)	GROUP NO. AND (NO. MICE CURED*) Sensitive Line	Melarsoprol - Resistant Line	Suramin - Resistant Line
Negative Control	0	1 (0)	12 (0)	24 (0)
Melarsoprol BG 80510	424	-	13 (0)	-
	106	-	14 (0)	-
	26.5	-	15 (0)	25 (3)
	6.63	2 (5)	16 (0)	26 (0)
	1.66	3 (5)	17 (0)	27 (0)
	0.414	4 (2)	-	28 (0)
	0.1035	5 (0)	-	29 (0)
	0.026	6 (0)	-	-
Suramin BH 58595	424	-	-	30 (0)
	106	-	-	31 (0)
	26.5	-	18 (5)	32 (0)
	6.63	7 (5)	19 (5)	33 (0)
	1.66	8 (4)	20 (1)	-
	0.414	9 (0)	21 (0)	-
	0.1035	10 (0)	22 (0)	-
	0.026	11 (0)	23 (0)	-

* No. mice alive 30 days after infection with T. rhodesiense.

TABLE VIII

Experiment #15

DAILY MORTALITY OF MICE TREATED WITH MELARSOPROL AND SURAMIN FOLLOWING INFECTION WITH THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Group No.	No. Mice Dead/Day Died	No. Mice Cured
1	5/4	0
2	-	5
3	-	5
4	1/7, 1/8, 1/16	2
5	2/6, 2/7, 1/9	0
6	2/6, 3/7	0
7	-	5
8	1/13	4
9	4/4, 1/5	0
10	5/4	0
11	5/4	0
12	5/4	0
13	5/4	0
14	5/4	0
15	1/4, 4/5	0
16	3/4, 2/5	0
17	1/4, 4/5	0
18	-	5
19	-	5
20	1/8, 1/9, 2/11	1
21	5/4	0
22	4/4, 1/5	0
23	4/4, 1/5	0
24	3/6, 2/11	0
25	1/14, 1/18	3
26	4/13, 1/14	0
27	2/5, 3/6	0
28	3/5, 2/6	0
29	1/5, 3/6, 1/7	0
30	2/6, 1/8, 1/17, 1/19	0
31	2/4, 3/5	0
32	2/4, 2/5, 1/6	0
33	2/4, 3/5	0

TABLE IX

DEVELOPMENT OF A SURAMIN-RESISTANT LINE OF *TRYPANOSOMA RHODESIENSE*

Passage No.	Dose (mg/kg)*
1-16	2
17-21	4
22-26	10
27-29	20
30-39	40
40-54	50
55-71	75
72-100	100
101-126	125
127-136	150
137-206**	100

* Drug administered subcutaneously.

** Beginning with passage no. 137, line passed twice weekly and given the indicated drug dosage to maintain drug resistance.

Note: Passages numbers 1-94 were made before the period of this annual report.

TABLE X

DEVELOPMENT OF A BERENIL-RESISTANT LINE OF TRYPANOSOMA RHODESIENSE

Passage No.	Dose (mg/kg)*
1-9	0.21
10-13	0.42
14-22	0.84
23-25	1.4
26-51	1.8
52-61	2.5
62-132	12.6

* Drug administered subcutaneously.

Note: Passages 1-12 were made before the period of this annual report.

TABLE XI

Experiment #9

COMPOUND CD_{50}* VALUES AND DEGREE OF RESISTANCE**
OF THE BERENIL-RESISTANT LINE OF TRYPANOSOMA RHODESIENSE
TO SELECTED TRYPANOSOMICIDAL COMPOUNDS

Drug	CD_{50} (mg/kg) Sensitive Line	CD_{50} (mg/kg) Berenil-Resistant Line	Degree of Resistance of Berenil-Resistant Line
Stilbamidine AH 55296	1.66	26.5	16-fold
Suramin BH 58595	6.63	6.63	1-fold
Melarsoprol BG 80510	1.66	106	64-fold
Berenil AH 78548	0.1035	26.5	256-fold
WR 163,577 BG 00521	1.66	>212	>128-fold

* CD_{50} is the minimal dose using at least 3 of 5 mice.

** Degree of resistance = CD_{50} of resistant line ÷ CD_{50} of sensitive line.

Note: All Compounds administered subcutaneously.

TABLE XII

Experiment #9

CURATIVE ACTIVITY OF SELECTED ANTITRYPANOSOMAL COMPOUNDS AT VARIOUS DOSES WHEN TESTED AGAINST THE DRUG-SENSITIVE AND BERENIL-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Drug	Dose (mg/kg)	GROUP NO. AND (NO. MICE CURED*) Sensitive Line	Berenil-Resistant Line
Negative Control	0	1 (0)	27 (0)
Stilbamidine	212	-	28 (5)
AH 55296	106	-	29 (5)
	53	-	30 (5)
	26.5	-	31 (5)
	6.63	2 (5)	32 (1)
	1.66	3 (4)	33 (0)
	0.414	4 (0)	-
	0.1035	5 (0)	-
Suramin	6.63	6 (5)	34 (3)
BH 58595	1.66	7 (1)	35 (0)
	0.414	8 (0)	36 (0)
	0.1035	9 (0)	37 (0)
Melarsoprol	106	-	38 (4)
BG 80510	26.5	-	39 (1)
	6.63	10 (5)	40 (0)
	1.66	11 (3)	41 (0)
	0.414	12 (0)	42 (0)
	0.1035	13 (0)	43 (0)
Berenil	212	-	44 (5)
AH 78548	106	-	45 (5)
	53	-	46 (5)
	26.5	-	47 (4)
	6.63	14 (5)	48 (0)
	1.66	15 (5)	49 (0)
	0.414	16 (5)	50 (0)
	0.1035	17 (3)	51 (0)
	0.026	18 (0)	52 (0)
WR 163,577	212	-	53 (0)
BG 00521	106	-	54 (0)
	53	-	55 (0)
	26.5	-	56 (0)
	6.63	19 (5)	57 (0)
	1.66	20 (5)	58 (0)
	0.414	21 (1)	59 (0)
	0.1035	22 (0)	60 (0)

TABLE XII
(continued)

Drug	Dose (mg/kg)	GROUP NO. AND (NO. MICE CURED*) Sensitive Line	Berenil-Resistant Line
Pentamidine	6.63	23 (5)	-
	1.66	24 (5)	-
	0.414	25 (1)	-
	0.1035	26 (0)	-

* Number of mice alive 30 days after infection with T. rhodesiense.

TABLE XIII

Experiment #9

DAILY MORTALITY OF MICE TREATED WITH ANTITRYPANOSOMAL COMPOUNDS FOLLOWING INFECTION WITH THE DRUG-SENSITIVE AND BERENIL-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Group No.	No. mice dead/day died	No. mice cured
1	5/4	0
2	-	5
3	1/21	4
4	1/5, 1/6, 1/10, 1/12, 1/21	0
5	5/4	0
6	-	5
7	1/4, 2/5, 1/14	1
8	5/4	0
9	5/4	0
10	-	5
11	2/14	3
12	1/5, 1/8, 2/12, 1/13	0
13	4/4, 1/6	0
14	-	5
15	-	5
16	-	5
17	1/12, 1/14	3
18	5/4	0
19	-	5
20	-	5
21	1/6, 1/11, 1/13, 1/17	1
22	5/4	0
23	-	5
24	-	5
25	1/5, 3/6	1
26	4/4, 1/11	0
27	5/4	0
28	-	5
29	-	5
30	-	5
31	-	5
32	2/4, 1/12, 1/21	1
33	1/5, 4/6	0

TABLE XIII
(continued)

Group No.	No. mice dead/day died	No. mice cured
34	1/14, 1/20	3
35	1/4, 1/5, 2/14, 1/17	0
36	5/4	0
37	5/4	0
38	1/14	4
39	1/9, 1/12, 2/15	1
40	2/4, 3/5	0
41	5/4	0
42	5/4	0
43	4/4, 1/5	0
44		5
45		5
46		5
47	1/17	4
48	1/4, 1/5, 1/6, 2/12	0
49	4/4, 1/5	0
50	5/4	0
51	3/4, 2/5	0
52	5/4	0
53	1/4, 3/5, 1/7	0
54	1/4, 4/5	0
55	5/5	0
56	1/4, 2/5, 1/6	0
57	1/4, 4/5	0
58	3/5, 1/6, 1/10	0
59	1/5, 4/6	0
60	1/4, 2/5, 2/6	0

TABLE XIV

Experiment #11

COMPOUND CD_{50}* VALUES AND DEGREE OF RESISTANCE**
OF THE BERENIL-RESISTANT LINE OF
TRYPANOSOMA RHODESIENSE TO SELECTED
TRYPANOSOMICIDAL COMPOUNDS

Drug	CD_{50} (mg/kg) Sensitive Line	Berenil-Resistant Line	Degree of Resistance of Berenil Resistant Line
WR 163,577 BG 00521	1.66	>212	>128 - fold
Pentamidine ZB 38055	0.414	6.63	16 - fold
Berenil AH 78548	0.1035	53	512 - fold
Stilbamidine AH 55296	0.414	53	128 - fold
Melarsoprol BG 80510	1.66	26.5	16 - fold

* CD_{50} is the minimal dose curing at least 3 of 5 mice.

** Degree of resistance = CD_{50} of resistant line ÷ CD_{50} of sensitive line.

Note: All compounds administered subcutaneously.

TABLE XV

Experiment #11

CURATIVE ACTIVITY OF SELECTED ANTITRYPANOSOMAL COMPOUNDS AT VARIOUS DOSES WHEN TESTED AGAINST THE DRUG-SENSITIVE AND BERENIL-RESISTANT LINE OF TRYPANOSOMA RHODESIENSE

Drug	Dose (mg/kg)	Group No. and (No. Mice Cured*) Sensitive Line	Berenil-Resistant Line
Negative Control	0	1 (0)	18 (0)
WR 163,577	212	-	19 (0)
BG 00521	53	-	20 (0)
	6.63	-	21 (0)
	1.66	2 (5)	22 (0)
	0.414	3 (2)	-
	0.1035	4 (0)	-
Pentamidine	212	-	23 (5)
ZB 38055	53	-	24 (5)
	6.63	-	25 (5)
	1.66	5 (5)	26 (2)
	0.414	6 (5)	27 (0)
	0.1035	7 (0)	-
Berenil	212	-	28 (5)
AH 78548	53	-	29 (5)
	6.63	-	30 (0)
	1.66	-	31 (0)
	0.414	8 (5)	-
	0.1035	9 (3)	-
	0.026	10 (0)	-
Stilbamidine	212	-	32 (5)
AH 55296	53	-	33 (4)
	6.63	-	34 (1)
	1.66	11 (5)	35 (0)
	0.414	12 (5)	36 (0)
	0.1035	13 (0)	-
Melarsoprol	212	-	37 (5)
BG 80510	53	-	38 (4)
	26.5	-	39 (4)
	6.63	14 (5)	40 (0)
	1.66	15 (5)	-
	0.414	16 (1)	-
	0.1035	17 (0)	-

* No. mice alive 30 days after infection with T. rhodesiense.

TABLE XVI

Experiment #11

DAILY MORTALITY OF MICE TREATED WITH SELECTED ANTITRYPANOSOMAL COMPOUNDS FOLLOWING INFECTION WITH THE DRUG-SENSITIVE AND BERENIL-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Group No.	No. mice dead/day died	No. mice cured
1	5/4	0
2	-	5
3	1/9, 2/20	2
4	5/5	0
5	-	5
6	-	5
7	4/5, 1/6	0
8	-	5
9	1/11, 1/12	3
10	3/4, 2/6	0
11	-	5
12	-	5
13	2/4, 2/5, 1/6	0
14	-	5
15	-	5
16	1/14, 2/17, 1/19	1
17	1/5, 2/11, 1/12, 1/13	0
18	5/4	0
19	1/4, 2/5, 1/6, 1/11	0
20	4/4, 1/5	0
21	5/4	0
22	5/4	0
23	-	5
24	-	5
25	-	5
26	2/5, 1/7	2
27	3/4, 2/5	0
28	-	5
29	-	5
30	1/4, 3/5, 1/17	0
31	3/4, 2/5	0

TABLE XVI
(continued)

Group No.	No. mice dead/day died	No. mice Cured
32	-	5
33	1/19	4
34	2/4, 1/5, 1/11	1
35	3/4, 2/5	0
36	4/4, 1/5	0
37	-	5
38	1/20	4
39	1/17	4
40	3/5, 1/6, 1/14	0

TABLE XVII

Experiment #14

THE CD_{50}* VALUES OF STILBAMIDINE AND BERENIL ADMINISTERED SUBCUTANEOUSLY AND TESTED AGAINST THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Drug	Route of Administration	CD_{50} (mg/kg) Sensitive Line	Stilbamidine-Resistant Line	Berenil-Resistant Line
Stilbamidine	S.C.	6.63	212	212
	Oral	>26.5	>212	-
Berenil	S.C.	0.414	106	106

* CD_{50} is the minimal dose curing at least 3 of 5 mice.

S.C. - Subcutaneous.

TABLE XVIII

Experiment #14

DEGREES OF RESISTANCE* OF DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE TO STILBAMIDINE AND BERENIL

Drug	Route of Administration	Degree of Resistance	
		Stilbamidine-Resistant Line	Berenil-Resistant Line
Stilbamidine	S.C.	32	32
	Oral	ND	-
Berenil	S.C.	256	256

* Degree of resistance = CD_{50} of resistant line ÷ CD_{50} of sensitive line.

S.C. - Subcutaneous.

ND - Cannot be determined from data.

TABLE XIX

Experiment #14

CURATIVE ACTIVITY OF STILBAMIDINE AND BERENIL AT VARIOUS DOSES WHEN TESTED AGAINST THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Drug	Dose (mg/kg)	Group No. and (No. mice cured*) Sensitive Line	Stilbamidine-Resistant Line	Berenil-Resistant Line
Negative Control	0	1 (0)	13 (0)	33 (0)
Stilbamidine Given S.C.	212	-	14 (3)	34 (5)
	106	-	15 (0)	35 (1)
	53	-	16 (0)	36 (1)
	26.5	-	17 (0)	37 (0)
	6.63	2 (4)	18 (0)	38 (0)
	1.66	3 (1)	19 (0)	39 (0)
	0.414	4 (0)	-	-
	0.1035	5 (0)	-	-
Berenil Given S.C.	212	-	20 (5)	40 (5)
	106	-	21 (4)	41 (3)
	53	-	22 (0)	42 (2)
	26.5	-	23 (0)	43 (0)
	6.63	-	24 (0)	44 (0)
	1.66	6 (5)	25 (0)	45 (0)
	0.41	7 (3)	26 (0)	-
	0.1025	8 (0)	-	-
	0.026	9 (0)	-	-
Stilbamidine Given Orally	212	-	27 (0)	-
	106	-	28 (0)	-
	53	-	29 (0)	-
	26.5	10 (0)	30 (0)	-
	6.63	11 (0)	31 (0)	-
	1.66	12 (0)	32 (0)	-

* No. mice alive 30 days after infection with T. rhodesiense.

S.C. - Subcutaneously.

TABLE XX

Experiment #14

DAILY MORTALITY OF MICE TREATED WITH STILBAMIDINE AND BERENIL FOLLOWING INFECTION WITH THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Group No.	No. mice dead/day died	No. mice cured
1	4/5	0
2	1/7	4
3	3/6, 1/7	1
4	2/4, 2/5, 1/6	0
5	5/4	0
6	-	5
7	1/12, 1/14	3
8	3/5, 1/7, 1/11	0
9	5/5	0
10	4/4, 1/5	0
11	4/4, 1/5	0
12	4/4, 1/5	0
13	2/4, 3/5	0
14	2/5	3
15	2/4, 3/5	0
16	1/4, 4/5	0
17	2/4, 3/5	0
18	1/4, 4/5	0
19	4/4, 1/5	0
20	-	5
21	1/12	4
22	5/5	0
23	1/4, 4/5	0
24	2/4, 3/5	0
25	1/4, 4/5	0
26	5/5	0
27	4/5, 1/11	0
28	4/4, 1/5	0
29	1/4, 3/5, 1/6	0
30	3/4, 2/5	0
31	4/4, 1/5	0
32	2/4, 3/5	0

TABLE XX
(continued)

Group No.	No. mice dead/day died	No. mice cured
33	4/4, 1/5	0
34	-	5
35	1/6, 1/11, 1/13, 1/14	1
36	3/5, 1/6	1
37	4/4, 1/5	0
38	5/4	0
39	5/4	0
40	-	5
41	2/11	3
42	1/5, 1/12, 1/17	2
43	2/4, 3/5	0
44	4/4, 1/5	0
45	3/4, 2/5	0

TABLE XXI

DEVELOPMENT OF A PENTAMIDINE-RESISTANT LINE OF TRYPANOSOMA RHODESIENSE

PASSAGE NO.	DOSE (mg/kg)*
1-9	0.28
10-11	0.56
12-13	1.12
14-16	2.8
17	5.6
18	8.3
19-25	20.0

* Drug administered subcutaneously.

TABLE XXII

DEVELOPMENT OF A STILBAMIDINE-RESISTANT LINE* OF TRYPANOSOMA RHODESIENSE

Passage Number	Dose (mg/kg)**
1-4	1.5
5-9	3
10-14	6
15-23	20
24-29	25
30-86	30
87-101	37.5
102-110	75
111-138	112.5
139-145	150

* This line discontinued at passage no. 145.

** Drug administered orally.

Note: Passages numbered 1-101 were made before the period of this annual report.

TABLE XXIII

DEVELOPMENT OF A STILBAMIDINE-RESISTANT LINE OF TRYPANOSOMA RHODESIENSE

Passage Number	Dose (mg/kg)*
1-4	0.125
5-8	0.25
9-13	0.5
14-18	1
19-29	2
30-44	4
45-48	6

* Drug administered subcutaneously.

Note: This line started after first stilbamidine-resistant line was discontinued at passage number 145.

TABLE XXIV

DEVELOPMENT OF LINES OF TRYPANOSOMA RHODESIENSE RESISTANT TO WR 163,577, STILBAMIDINE, AND A COMBINATION OF WR 163,577 AND STILBAMIDINE

Resistant Line	Passage Number	Dose (mg/kg)*
WR 163,577 BG 00521	1-7	0.125
	8-15	0.25
	16	0.5
Stilbamidine AH 55296	1-7	0.125
	8-15	0.25
	16	0.5
Combination:		
WR 163,577 (a) + Stilbamidine (b)	1-7	0.0625(a) + 0.0625(b)
	8-15	0.125(a) + 0.125(b)
	16	0.25(a) + 0.25(b)

* Compound administered subcutaneously.

Note: Passages number 1-12 were made before the period of this annual report.
Lines discontinued after passage number 16.

TABLE XXV

THE CD_{50}* VALUES OF WR 163,577 AND STILBAMIDINE ADMINISTERED SUBCUTANEOUSLY AND TESTED AGAINST THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Drug	CD_{50} (mg/kg)*			
	Sensitive Line	Resistant Lines		
		WR 163,577	Stilbamidine	Combination**
WR 163,577 BG 00521	0.25	>256	>256	>256
Stilbamidine AH 55296	0.125	16	64	64

* CD_{50} is the minimal dose curing at least 3 of 5 mice.

** WR 163,577 and stilbamidine (1:1).

TABLE XXVI

DEGREES OF RESISTANCE* OF DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE TO WR 163,577 AND STILBAMIDINE

Drug	DEGREE OF RESISTANCE		
	WR 163,577 - Resistant Line	Stilbamidine- Resistant Line	Combination** Resistant Line
WR 163,577 BG 00521	>1024	>1024	>1024
Stilbamidine AH 55296	128	512	512

* Degree of resistance = CD_{50} of resistant line ÷ CD_{50} of sensitive line.

** WR 163,577 and stilbamidine (1:1).

TABLE XXVII

CURATIVE ACTIVITY OF WR 163,577 AND STILBAMIDINE AT VARIOUS DOSES WHEN TESTED AGAINST THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF TRYPANOSOMA RHODESIENSE

Drug	Dose (mg/kg)	Group No. and (No. of mice cured*) Sensitive Line	Resistant Lines WR 163,577	Stilbamidine	Combination**
Negative Control	0	1 (0)	10 (0)	18 (0)	26 (0)
WR 163,577	256	-	11 (1)	19 (1)	27 (0)
BG 00521	64	-	12 (0)	20 (0)	28 (0)
	16	-	13 (0)	21 (0)	29 (0)
	4	2 (5)	-	-	-
	1	3 (4)	-	-	-
	0.25	4 (3)	-	-	-
	0.125	5 (2)	-	-	-
Stilbamidine	256	-	14 (5)	22 (5)	30 (5)
AH 55296	64	-	15 (5)	23 (3)	31 (5)
	16	-	16 (3)	24 (2)	32 (2)
	4	6 (5)	17 (0)	25 (0)	33 (0)
	1	7 (4)	-	-	-
	0.25	8 (5)	-	-	-
	0.125	9 (3)	-	-	-

* Number of mice alive 30 days after infection.

** WR 163,577 and stilbamidine (1:1).

TABLE XXVIII

DAILY MORTALITY OF MICE TREATED WITH WR 163,577 AND STILBAMIDINE FOLLOWING INFECTION WITH THE DRUG-SENSITIVE AND DRUG-RESISTANT LINES OF *TRYPANOSOMA RHODESIENSE*

Group No.	No. mice dead/day died	No. mice cured
1	5/4	0
2	-	5
3	1/15	4
4	1/10, 1/11	3
5	1/7, 1/11, 1/13	2
6	-	5
7	1/27	4
8	-	5
9	1/5, 1/10	3
10	5/4	0
11	1/4, 1/9, 1/13, 1/18	1
12	1/4, 1/5, 1/7, 1/10, 1/11	0
13	4/4, 1/5	0
14	-	5
15	-	5
16	1/7, 1/9	3
17	2/5, 2/6, 1/9	0
18	5/4	0
19	1/4, 1/11, 1/17, 1/21	1
20	2/4, 3/5	0
21	4/4, 1/5	0
22	-	5
23	1/10, 1/17	3
24	1/5, 2/9	2
25	3/4, 2/5	0
26	5/4	0
27	1/4, 1/6, 1/8, 1/12, 1/13	0
28	4/4, 1/6	0
29	4/4, 1/6	0
30	-	5
31	-	5
32	1/5, 1/10, 1/17	2
33	3/4, 2/5	0

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